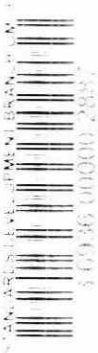

HAZARDOUS CONTAMINANTS PROGRAMME

POLYNUCLEAR AROMATIC HYDROCARBONS

– A Background Report Including Available Ontario Data –

ARB – TDA – Report No. 58–79

September 1979

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1979

Ontario
Ministry of the Environment

The Honourable
Harry C. Parrott, D.D.S.,
Minister

Graham W. S. Scott,
Deputy Minister

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- A Background Report Including
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Ministry of the Environment,
Air Resources Branch,
Technology Development
& Appraisal Section.

September, 1979

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EXECUTIVE SUMMARY AND MAIN CONCLUSIONS

1. The present review of environmental aspects of polynuclear aromatic hydrocarbons (PAH) has been undertaken as a prerequisite for possible regulatory action in view of the reported carcinogenic properties of a number of individual PAH compounds and of industrial mixtures containing them. As a result, some important information gaps have been identified and a number of conclusions may be reached.

2. Review of the physical properties of this large group of ubiquitous environmental pollutants formed during incomplete combustion of organic matter is consistent with the finding that they usually occur in the solid state, mainly adsorbed on the surface of particulate matter.

   - However, the possibility of their volatilization and sublimation in atmospheric environment, and their solubilization by natural and man-made surface-active agents in aqueous environment has to be taken into account when sampling is carried out in these media.

   - One environmentally important physical characteristic of PAH compounds is their tendency, as shown by actual Ontario data, to be associated with the respirable fraction of airborne particulate matter. Other physical factors, such as shape, surface area and other surface characteristics of PAH-containing particulates may have equally important health implications and should receive closer scrutiny.

3. The characteristic molecular structures of PAH compounds, comprising multiple fused benzene rings with extensively conjugated electronic systems, determine, to a large measure, their chemical and biological activities and give rise to their characteristic ultraviolet and luminescence spectra.

PAH are generally more reactive than simple benzene derivatives and undergo all the characteristic chemical reactions of aromatic systems, including substitutions, additions and eliminations. Of particular environmental significance are their photooxidation reactions as well as their reactions with ozone, nitrogen oxides, sulfur oxides, nitric and sulfuric acids.

- Further research aimed at elucidating reaction mechanisms and identifying end-products of little-studied PAH compounds is clearly indicated. The possible generation of highly reactive, so-called singlet oxygen during oxidation reactions of PAH also needs further investigation.

Covalent binding of PAH to proteins and nucleic acids and particularly to DNA is generally thought to be a prerequisite of their biological activity. However, for such binding to occur, PAH must first be chemically modified, or activated, through enzymatic mechanisms in biological systems. Among the large number of possible reactive PAH-derivatives the so-called Bay-region diol epoxides have recently emerged as the most likely ultimate carcinogenic metabolites of PAH.

4. Formation of PAH occurs during incomplete combustion of almost any kind of organic matter, as a result of pyrolytic and pyrosynthetic processes, the optimum temperature range being situated between 650°C and 850°C. Natural sources of PAH include forest fires and volcanic eruptions as well as the possibility of formation by biosynthesis and by low-temperature processes involving decaying organic matter. Anthropogenic sources of PAH are mainly due to the intentional burning of solid or liquid fossil fuels for industrial, heating, cooking or transportation purposes.

Despite considerable uncertainty, estimation of Ontario emissions has been attempted in terms of annual emissions of Benzo(a)pyrene from potential sources, based on emission factors developed by the U.S. Environmental Protection Agency and on available statistics regarding Ontario production or fuel consumption data.

- These calculated emission estimates indicate that forest fires, along with other uncontrolled burning of organic matter probably contribute at least as much to the total environmental burden of PAH in Ontario as the major industrial and mobile sources, providing justification for increased monitoring and regulatory activity in these areas.

- Cigarette smoke, including the so-called side-stream smoke, is the single most significant source of PAH in terms of personal exposure and impact. Local regu-

lations aimed at preventing contamination of enclosed public areas with side-stream cigarette smoke should therefore be encouraged.

- Among industrial sources, the production of metallurgical coke is the single most significant potential source of ambient PAH pollution in Ontario, as shown by potential B(a)P emission of 9.3 Mg per year. Due to the fugitive nature of most coke oven emissions, reduction of particulate emissions through use of best available control technology seems to be the most logical approach to regulatory control.
- Heat and power generation as well as municipal and commercial incinerators, with a combined total of annual B(a)P emissions estimated at 332 kg, are other potentially significant sources of PAH in Ontario. Control of PAH formation and emission in these cases is best achieved through promotion of good maintenance and efficient combustion techniques, in addition of using appropriate pollution control equipment. Additional source testing may also be advisable in some cases.

- Motorcycles and snowmobiles, powered by 2 -cycle engines, have to be singled out as the major polluters among mobile sources of PAH. These vehicles lack adequate emission control devices and it is felt that requiring installation of such devices would be desirable.

5. Photooxidation, as well as reactions with ozone, hydroxyl radicals, nitrogen oxides and sulfur dioxide are the possible means of chemical degradation of PAH in the atmosphere. Quinones and acidic decomposition products have been observed in a number of cases, as the result of PAH degradation processes. The available experimental evidence indicates that chemical half-lives of PAH should be limited to a few hours or days at most upon direct exposure to the abovementioned reactants. However, due to the shielding effect of multi-layering and particle agglomeration, PAH have been shown to survive relatively long (up to 40 days) atmospheric residence times without significant degradation, their physical removal occurring through the usual processes of dry and wet deposition.

Photooxidation also appears to be the principal means of chemical degradation of PAH in natural water systems. Half-lives in aqueous systems have only been reported for Benzanthracene and Benzo(a)pyrene, ranging from 1.6 hours to 15 days under various laboratory conditions. However, most PAH are expected to be quite stable in deep, cold, turbid waters and bottom sediments under anaerobic conditions and in the absence of light. Besides photooxidation, elimination of PAH from natural water bodies may occur through sedimentation as well as by bioaccumulation and biodegradation.

Elimination of PAH from drinking water supplies may be achieved by physical means such as sedimentation and filtra-

tion through activated charcoal, as well as chemically by chlorination or ozonation.

- Chlorination and ozonation products of PAH are not well known and further study of their nature, properties and effects is felt desirable.

Photooxidation and biodegradation by some soil microorganisms may be an effective means of natural disposal of PAH in surface layers of earth. However, in the absence of sunlight and of PAH-degrading bacteria they are expected to persist in soil for long periods of time.

- In general, data regarding the environmental persistence and fate of a number of individual PAH compounds are lacking and the need for further research in this area is evident.

6. The reported carcinogenicity of a number of PAH in animal experiments is the main cause of environmental concern. However, at least an equally large number of PAH were shown to be inactive in animal experiments and, although occupational exposure to complex PAH-containing industrial mixtures has been recognized as a cause of human cancer, no single PAH compound has so far been proven to cause cancer in humans under normal environmental conditions.

Accurate or even approximate estimation of the carcinogenic risk represented by exposure to ambient levels of PAH is made exceedingly difficult by a number of complicating factors, including the extreme complexity of the carcinogenic process itself, the individual and interspecies differences in metabolic activation, the uncertainties involved in high-to-low-dose extrapolations and the existence of co- and anticarcinogenic effects due to the presence of other chemicals in the environment.

- Despite these various difficulties in deriving unequivocal quantitative estimates of magnitudes of risk, the available evidence based on epidemiological data in both occupational and ambient settings nevertheless points to the reality of such risk and to the importance of keeping PAH emissions at the lowest levels possible.

A large number of animal tests, both in-vivo and in-vitro, have been developed and applied for screening of carcinogenic potential of PAH compounds and results of these studies and reviewed in the main body of this report. Results obtained through some of the in-vitro methods, including several mutagenesis test systems, are in good agreement with those provided by the more costly and time-consuming in-vivo methods.

- Further study of metabolism, pharmacokinetic and structure-activity relationships, continuing efforts at developing improved, more predictive, faster and less expensive carcinogenic screening tests and the use of the latter in conjunction with more comprehensive chemical analysis will undoubtedly provide a better basis for estimating the extent of cancer risk due to ambient environmental exposures to PAH.

7. Although other toxic effects of PAH are certainly overshadowed by their carcinogenic potential, such effects should not be left unnoticed. Such toxic manifestations include enzyme induction and immunosuppression as well as various respiratory and skin disorders in humans and in experimental animals. Various adverse effects have been reported in invertebrates, fish and amphibian species as well, indicating possible ecological consequences.

- While bioaccumulation of PAH has been demonstrated in several aquatic organisms and plants, it is not expected to present nearly as serious a problem as organochlorine pesticides for instance, as they are metabolized and biodegraded much more efficiently by various strains of bacteria and fish. Nevertheless, this is an area of study requiring further investigation.

8. To meet the challenge presented by the great complexity of PAH-containing environmental mixtures a number of efficient analytical techniques have been developed for their extraction, clean-up, separation and quantitative determination.

Sampling devices include various modifications of the EPA Method 5 sampling train for source sampling, high-volume samplers using glass fiber filters for ambient air sampling, as well as various types of adsorbent cartridges for personal and water sampling.

- The use of back-up adsorbents is desirable in order to avoid loss of vapor-phase PAH during sampling. Furthermore, collection of size-fractionated samples serves to better characterize them with regard to potential health implications.
- Extraction of PAH from the sampling matrix may be accomplished by solvent treatment, thermal desorption or vacuum sublimation. Whatever the method chosen, the efficiency of extraction from the sampling medium must be determined for each particular compound if accurate quantitative determination of that compound is desired.

Separation of PAH from other classes of interfering impurities may be achieved by various clean-up procedures, including acid-base extraction, solvent partitioning and column chromatography. Some of the

original PAH present may be lost at this stage as well, and the extent of such losses must also be taken into account in the final analysis.

Following initial clean-up of sample extracts, the techniques of thin-layer chromatography (TLC), gas chromatography (GC) or high-pressure liquid chromatography (HPLC) may be used for analytical separation of individual PAH compounds, including isomers. Each of these techniques has some advantages to offer, including highest speed and lowest cost in the case of TLC, best resolution in the case of GC and highest sensitivity in the case of HPLC. The ultimate choice depends of course upon the particular analytical goal to be achieved.

A number of efficient methods are also available for the final identification and quantitative determination of individual PAH compounds. These include ultra-violet-visible spectrophotometry, fluorimetry, mass spectrometry as well as the use of flame ionization, photoionization and electron capture detectors in gas chromatography. Lowest limit of detection achieved to-date, using HPLC separation and fluorimetric determination, is in the low picogram range.

- The very diversity of sampling, clean-up and analytical methods available for the detection and quantitation of PAH makes detailed reporting of methods used in each case necessary, and eventual standardization of methodology desirable. Furthermore, the presently available analytical methods, while adequate in sensitivity, accuracy and reproducibility, are nevertheless not simple and economical enough for routine, widespread field monitoring purposes and development of such methods is still required.

9. To date , over 100 different PAH compounds have been detected in airborne particulate matter and other environmental samples. Ambient atmospheric levels of PAH generally range from less than 1 ng/m^3 to about 20 ng/m^3. B(a)P has traditionally been used as an indicator for other PAH present and although this practice is questionable, it does provide useful information with regard to general trends in PAH levels. Thus, urban B(a)P levels have shown a decreasing trend over the past decade, annual average levels dropping from about 6 ng/m^3 to about 1 ng/m^3 in North America. Available Ontario data are also consistent with this trend. However, there is still a noticeable difference between urban and rural levels, while the increased winter vs. summer values are much less evident than they were a decade ago. The presence of PAH has been detected in water, soil and various raw and processed foodstuffs as well, at levels ranging from a few parts per

trillion to parts per million, depending on the degree of contamination.

- Little or no Ontario data are available regarding PAH levels in water, soil and food and development of programs in this area, as well as continued monitoring of ambient atmospheric levels is felt desirable.

10. Current regulations related to PAH are directed towards the control of complex environmental mixtures that are known to contain them, rather than towards the control of individual PAH compounds themselves. Thus, occupational exposures are usually controlled in terms of coal-tar-pitch volatiles, where a threshold limit value of 0.2 mg/m^3 has been adopted by a number of countries and by Ontario as well.

Protection of the general population against excessive exposure to ambient atmospheric PAH is thought to be achieved through control of total suspended particulate matter, with annual maximum geometric mean of 60 $\mu g/m^3$ being called for in Ontario. Finally, while the World Health Organization has recommended a limit of 30 ng/L for total carcinogenic PAH in drinking water, such standards have yet to be officially adopted in many countries, including Canada and the U.S.

- Future alternatives in regulating environmental PAH levels include better control over particulates in the respirable size range, requiring the installation and use of best available control and/or treatment

technology and development of compound-specific regulatory standards. However, before the latter goal can be achieved it is felt that more definite and specific correlation has to be established between quantitative chemical composition and biological activity of complex, PAH-containing environmental mixtures through simultaneous chemical and biological monitoring.

INTRODUCTION

Polynuclear Aromatic Hydrocarbons (PAH) have been identified as one of several groups of potentially hazardous environmental contaminants by the Hazardous Contaminants Technical Committee of the Ontario Ministry of the Environment. Study of their properties and effects was assigned high priority, as part of the Province's Hazardous Contaminants Programme.

Polynuclear (also known as polycyclic) Aromatic Hydrocarbons are a class of organic chemicals containing multiple fused benzene rings in their molecular structure. They are being formed through natural as well as anthropogenic processes, whenever and wherever incomplete combustion of carbonaceous matter, such as oil, wood, coal, gas or other organic matter, is taking place. Thus, PAH were present in the environment for many thousands of years before increasing levels due to human industrial activity began to give rise to concern regarding their potential hazards to human health.

The fact that long-term occupational exposure to mixtures containing these compounds may lead to various forms of cancer has been known since 1775, when Sir Percival Pott reported a high incidence of scrotal cancer among chimney sweeps, and has been well documented since then among workers exposed to soots, tars, oils and coke oven emissions (1 - 7). However, no direct causal relationships have been established between ambient environmental concentrations of specific Polynuclear Aromatic Hydrocarbons and increased occurrence of cancer, despite the fact that significant differences have been observed both in lung cancer rates and ambient Benzo(a)pyrene concentrations between urban and nonurban areas (8). Benzo(a)pyrene is considered to be one of the most

potent carcinogens of the PAH class of compounds, based on animal experiments. It has been the most extensively studied member of the group, both from chemical and biological viewpoints. Up to a few years ago, lack of suitable analytical techniques restricted ambient environmental measurements to a few selected PAH including Benzo(a)pyrene and ambient concentrations of this single compound became generally accepted as indicative of environmental contamination by Polynuclear Aromatic Hydrocarbons.

In actual fact however the situation is considerably more complex. The general class of Polynuclear Aromatic Hydrocarbons includes hundreds of compounds with widely varying physical and chemical properties and biological activities. Newer, more powerful analytical techniques are revealing the extreme complexity of PAH mixtures found in the environment. On the other hand, newer, more sensitive and specific biological testing methods allow us a deeper insight into the mechanism of their activity. Physicochemical interactions of various PAH with each other, with other environmental components and with biological systems may enhance, inhibit or modify their carcinogenic potential to varying degrees.

In order to assess the potential hazards to human health resulting from ambient exposure to Polynuclear Aromatic Hydrocarbons and in order to derive meaningful decisions as to the necessity and strategies for control measures, one has to review their physical and chemical characteristics, identify their potential sources and the available abatement techniques, evaluate sampling and analytical techniques, examine their fate and persistence in the environment, consider their biological effects and suggested mechanisms of activity, present the available environmental con-

centrations and trends, and summarize existing standards and other regulatory control measures.

Several excellent reviews have appeared in the past few years relating to this general subject matter (9 - 17), and the reader is referred to these for additional information. This report attempts to present an up-to-date overview of the current state of knowledge regarding the environmental aspects of Polynuclear Aromatic Hydrocarbons. The related nitrogen-containing aza-arenes are not included in the scope of this report.

1. PHYSICAL AND CHEMICAL PROPERTIES OF PAH

1.1 Physical Properties:

1.1.1 General:

Polynuclear Aromatic Hydrocarbons are generally high melting, high boiling solids, with extremely low water solubility and low vapor pressure at ambient temperature and atmospheric pressure. The chemical structures and basic physical properties of a few representative carcinogenic and non-carcinogenic PAH of environmental importance are summarized in Tables I - IV, based on references 9,12,16,18 and 19. The reader is referred to these and to reference 14 for a more complete list of PAH and aza-arenes along with their respective carcinogenic activities. It should be pointed out at the outset that the terms "carcinogenic" and "non-carcinogenic" do not categorize these compounds unequivocally. Compounds classified as "carcinogenic", based on animal experiments, may not necessarily cause cancer in humans. Conversely, compounds classified as "non-carcinogenic", based on insufficient or inadequate data, may later turn out to be carcinogenic in humans. Classification of PAH according to their biological effects will be discussed in more detail in Chapter 4.

In the following paragraphs we shall review those physical properties of PAH which are the most significant ones in determining their environmental fate, their chemical and biological activity, and which are relevant to their analytical methodology, namely their physical state and size-distribution patterns in airborne particulates, their solubility and solubilization, their electronic structure and their spectral properties.

TABLE I

SOME "NON-CARCINOGENIC" POLYNUCLEAR AROMATIC HYDROCARBONS DETECTED IN POLLUTED ENVIRONMENTS

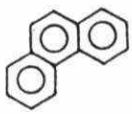
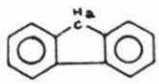
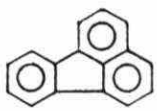
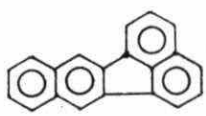
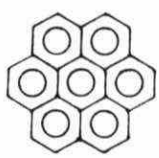
COMPOUND NAME	ABBREVIATION	STRUCTURE	MOL. FORMULA	MOL. WT.
ANTHRACENE	A		$C_{14}H_{10}$	178.22
PHENANTHRENE	Phen		$C_{14}H_{10}$	178.22
FLUORENE	Fln		$C_{13}H_{10}$	166.2
PYRENE	P		$C_{16}H_{10}$	202.24
FLUORANTHENE	F		$C_{16}H_{10}$	202.24
BENZO(k)FLUORANTHENE	B(k)F		$C_{20}H_{12}$	252.32
PERYLENE	Per		$C_{20}H_{12}$	252.32
BENZO(g,h,i)PERYLENE	B(g,h,i)Per		$C_{22}H_{12}$	276.34
CORONENE	Cor		$C_{24}H_{12}$	300.36
DIBENZO(cd,jk)PYRENE (ANTHANTHRENE)	DB(cd,jk)P		$C_{22}H_{14}$	278.35

TABLE II - PHYSICAL PROPERTIES OF SOME "NON-CARCINOGENIC" PAH DETECTED IN POLLUTED ENVIRONMENTS

COMPOUND	APPEARANCE	SOLUBILITY PROFILE	MELTING POINT (°C)	BOILING POINT °C, 1 atm.	DENSITY	VAPOR PRESSURE at 25°C (Torr)	EQUILIBRIUM VAPOR CONCENTRATION AT 25°C (ng/l)
A	Colorless, monoclinic Plates, with violet fluorescence	Insoluble in water, soluble in EtOH(1:67) CS_2 (1:31) MeOH(1:70) Cl_4 (1:86) Bz(1:62) $CHCl_3$(1:85)	218 (Sublimes)	342	1.25^{27}_4	1.95×10^{-4}	1.87×10^{7}
Phen	Colorless, monoclinic plates	Practically insoluble in water Soluble in: Toluene (1:2.4) CS_2(1:1) Bz(1:2) Et_2O(1:3) CCl_4(1:2.4)	100 (Sublimes in high vacuum)	340	1.179^{25}_4	6.8×10^{-4}	6.51×10^{7}
Fln	White leaflets	Almost insoluble in water. Soluble in CS_2, Ether, Acetone, Bz, Acetic Acid.	116-117 (Sublimes in high vac)	293-295	1.203°_4		
P	Pale yellow platelets with slight blue fluorescence	Almost insoluble in water. Sol. in alcohol, ether, benzene, CS_2	156	404	1.271^{23}_4	6.85×10^{-7}	7.4×10^{4}
F	Pale yellow needles or platelets	Almost insoluble in water. Soluble in most organic solvents.	110-111	393	1.252°_4		
B'k)F	Pale yellow needles	Almost insoluble in water. Soluble in alcohol, benzene, acetic acid.	216-217	480		9.59×10^{-11}	13
Per	Golden brownish-yellow plates	Almost insoluble in water. Slightly sol. in ether, alcohol, acetone. Sol. in benzene, very sol. in $CHCl_3$ and CS_2.	273-274	Sublimes between 350-400	1.35		
B(g,h,i)Per	Pale, yellow-green plates from xylene.	Almost insoluble in water. Slightly soluble in benzene, ethanol.	277			1.01×10^{-10}	1.5
Cor	Yellow needles	Insoluble in water. Slightly soluble in benzene.	438-440	525	1.377	1.47×10^{-12}	0.02
DB(cd,jk)P	Golden yellow plates from xylene	Insoluble in water. Slightly soluble in benzene.	261				

TABLE III

SOME "CARCINOGENIC" POLYNUCLEAR AROMATIC HYDROCARBONS DETECTED IN POLLUTED ENVIRONMENTS

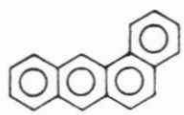
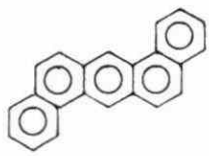
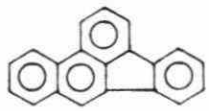
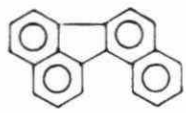
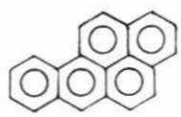
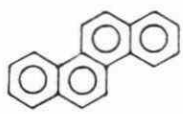
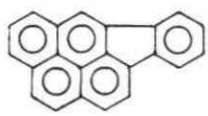
COMPOUND NAME	ABBREVIATION	STRUCTURE	OTHER NAMES	MOL. FORMULA	MOL. WT.
BENZ(a)ANTHRACENE	B(a)A		1,2-Benzanthracene	$C_{18}H_{12}$	228.28
DIBENZ(a,h)ANTHRACENE	DB(a,h)A		1,2-5,6-Dibenzanthracene	$C_{22}H_{14}$	278.33
BENZO(b)FLUORANTHENE	B(b)F		Benz(e)acephenanthrylene 2,3-Benzofluoranthene	$C_{20}H_{12}$	252.32
BENZO(j)FLUORANTHENE	B(j)F		7,8-Benzofluoranthene 10,11-Benzofluoranthene	$C_{20}H_{12}$	252.32
BENZO(a)PYRENE	B(a)P		1,2-Benzpyrene (3,4-Benzpyrene)	$C_{20}H_{12}$	252.32
BENZO(e)PYRENE	B(e)P		4,5-Benzpyrene (1,2-Benzpyrene)	$C_{20}H_{12}$	252.32
CHRYSENE	Chry		1,2-Benzophenanthrene Benz(a)phenanthrene	$C_{18}H_2$	228.28
INDENO(1,2,3,-cd)PYRENE	IP		2,3-o-Phenylene-pyrene	$C_{22}H_{12}$	286.34

Table III(cont'd)

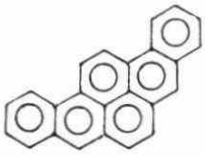
COMPOUND NAME	ABBREVIATION	STRUCTURE	OTHER NAMES	MOL. FORMULA	MOL WT.
DIBENZO(a,h)PYRENE	DB(a,h)P		Dibenzo(b,def)-chrysene	$C_{24}H_{14}$	302.38
DIBENZO(a,i)PYRENE	DB(a,i)P		Benzo(r,s,t)pentaphene 2,3-6,7-Dibenzpyrene	$C_{24}H_{14}$	3-2/39

TABLE IV - PHYSICAL PROPERTIES OF SOME "CARCINOGENIC" PAH DETECTED IN POLLUTED ENVIRONMENTS

COMPOUND ABBREVIATION	APPEARANCE	SOLUBILITY PROFILE	MELTING POINT (^{o}C)	BOILING POINT (^{o}C, 1 atm)	DENSITY	VAPOR PRESSURE at 25^{o}C (Torr)	EQUILIBRIUM VAPOR CONCENTRATION AT 25^{o}C (ng/m^{3})
B(a)A	Colourless leaflets or plates, with yellowish green (to violet) fluorescence.	Almost insoluble in H_2O. Difficulty soluble in boiling alcohol. Soluble in most other organic solvents.	159-161 (Sublimes)	400		1.1×10^{-7}	1333
DB(a,h)A	Colourless plates or leaflets from acetic acid.	Almost insolbule in H_2O. Slightly soluble in alcohol, ether. Soluble in most other organic solvents.	266-267 (Sublimes)		1.282		
B(b)F	Colourless needles from benzene, toluene or acetic acid.	Almost insoluble in H_2O. Slight soluble in alcohol.	167-168				
B(j)F	Yellow plates or needles from alcohol.	Almost insoluble in H_2O Slightly soluble in alcohol.	165-166				
B(a)P	Pale yellow needles or plates from benzene/methanol.	Almost insoluble in H_2O. Sparingly soluble in ethanol, methanol. Soluble in benzene, toluene, xylene.	176.5-177.5	475	1.351	5.49×10^{-9}	75
B(e)P	Colourless prisms or plates from alcohol or benzene.	Almost insoluble in H_2O. Soluble in aromatic solvents, cyclohexane, nitromethane.	178-179	492		5.54×10^{-9}	75
Chry	Colourless platelets with blue fluorescence.	Insoluble in H_2O. Slightly soluble in ethanol, ether, CS_2, gl. acetic acid. Moderately sol. in boiling B_z.	255.8-256.3 (Sublimes in vacuo)	448	1.274_4^{20}		
IP	Yellow plates or needles from light petroleum.	Almost insoluble in H_2O. Soluble in aromatic solvents, cyclohexane	162.5-164				
DB(a,h)P	Golden yellow plates from xylene or trichlorobenzene.	Almost insoluble in H_2O. Soluble in hot aromatic solvents.					
DB(a,i)P	Greenish-yellow needles, prisms or lamellae	Insoluble in H_2O. Almost insoluble in alcohol and other 1 part is sol. in 500 parts of boiling gl. acetic acid and 200 parts of boiling benzene.					

1.1.2. Physcial State and Size-Distribution Pattern of PAH in Airborne Particulates:

Formation of PAH in combustion processes is generally thought to occur in the vapor phase (20,21). Due to their generally high melting and boiling points and low vapor pressures at ambient temperatures, condensation and adsorption on airborne particulate matter occurs shortly upon cooling and it is believed that the overwhelming majority of atmospheric PAH is associated with aerosols (12). However, the existence and relative importance of vapor phase PAH in the atmospheric environment still remains a somewhat controversial and unresolved issue. Thus, based on experiments involving drawing filtered air through pure liquid paraffin for 38 days and examining the medium for fluorescence, COMMINS (22) concluded that no measurable amount of PAH exists in the vapor phase at ambient temperatures. The same conclusion was reached by THOMAS, MUKAI and TEBBENS (23), based on quantitative combustion experiments. However, the same authors (23) did collect a small amount of gaseous B(a)P at more elevated (80-90^{o}C) temperatures and RONDIA (24) observed relatively high volatilities of PAH in the presence of air-flow. Based on careful measurement of vapor pressures using the Knudsen weight loss effusion method (25), PUPP, LAO, MURRAY and POTTIE (20) derived equilibrium vapor concentrations of some PAH as a function of temperature and concluded that vapor concentrations of PAH during emission from sources are probably of the order of the calculated equilibrium vapor concentrations regardless of the presence of soot or other particulates. The same authors also gave words of caution about possible collection inefficiency using particulate

filters as well as about sublimation losses during storage or vacuum drying of PAH samples, as all these phenomena are proportional to the equilibrium vapor concentrations and may be significant for all but the most involatile compounds. The conclusions of PUPP, LAO, MURRAY and POTTIE regarding the presence of PAH compounds as vapors within the emission system is supported by recent theoretical calculations of NATUSCH and TOMKINS (26), who, incidentally, also predict that PAH vapors will be rapidly and quantitatively adsorbed onto the surfaces of airborne particulate matter at, or close to, the point of exit from the emission system. Thus,the majority of evidence suggests that under conditions of ambient temperatures and pressures the bulk of PAH compounds exist in the solid state, mainly adsorbed on the surface of particulate matter. Although the mechanism of adsorption has not been studied extensively, indications are (23) that adsorption occurs through hydrogen bonding to oxygen functional groups situated on the surface of soot and other particulate matter. This conclusion was reached based on observation of the lack of open-channel porosity, the large concentration of oxygen functional groups on the surface of soot particles, the relatively easy extractability and the rapid rate of photomodification of B(a)P associated with soot.

The distribution of PAH according to the particle size of airborne particulate matter with which they are associated is an area of study whose importance became recognized only in recent years. Of particular significance from the point of view of possible health hazards is the fact that the bulk of PAH is associated with

the respirable fraction of airborne particulate matter. This has been demonstrated by several authors: DeMAIO and CORN (27), in a study of particulates in Pittsburgh, Pa., found that more than 75% of B(a)P and several other PAH were associated with aerosols less than 2.5 μm in radius; KERTESZ-SARINGER and co-workers (28) showed that in Budapest 70 to 90% of the B(a)P-containing particles had a radius of 1 μm or less; ALBAGLI, OJA and DUBOIS (29), in a study of PAH-containing airborne particulates collected in a large Canadian city, reported that the average mass median diameter of B(a)P-containing particles was 0.64 μm and thus may be deposited deep in the alveoli of the respiratory tract. More recently, PIERCE and KATZ (30) found 70-90% of total PAH content associated with particles of less than 5 μm aerodynamic diameter, collected in Toronto. Essentially similar results were also obtained by DE WIEST and DELLA FIORENTINA (32) in Belgium. These experimental findings received theoretical support in a recent study by NATUSCH and TOMKINS (26), who concluded that adsorption of PAH at a given temperature occurs preferentially on the surface of smaller particles. It is speculated (12) that the determinating factor in limiting the size of the smallest PAH-containing particles might be the so-called Kelvin effect, according to which the equilibrium vapor pressure varies with the radius of curvature of the particle and with the surface energy of the volatile material. Unfortunately, calculation of the Kelvin effect in the case of PAH has so far been impossible due to the absence of available data regarding the relevant surface energies.

In addition to determining the respirable nature of PAH-containing aerosols, indications are that particle size also determines the rate of elution of PAH in biological media. Thus, FALK et al. (33) and KUTSCHER et al (34) found that the elution rate is considerably reduced from soot particles less than 0.04 um in diameter. Factors such as surface area and shape of the ambient particles might also play a significant role in determining bio-availability of PAH (35), but this is a very little studied area, and much more additional information is needed to evaluate the influence of particulate surface characteristics on the biological availability and activity of PAH.

1.1.3. Solubility and Solubilization:

As indicated in Tables II and IV, PAH have very low intrinsic solubility in water. Figures given by DAVIS and co-workers (36) are 5×10^{-4} mg/L, 4×10^{-3} mg/L and 11×10^{-3} mg/L at 27°C for DB(a,h)A, B(a)P and BA respectively. More recent measurements by MACKAY and SHIU(443) essentially confirm these data. It is estimated(16) that water solubilities of most PAH with more than three rings in their molecular structure are of the order of 10^{-10} moles/L or less. In contrast, PAH exhibit much higher solubilities in aromatic and other organic solvents, as illustrated for example by values of octanol-water partition coefficients of 10^4-10^6 determined by HANSCH and co-workers (16, p. 15). These values tend to indicate that significant bio-accumulation of PAH might occur in fatty tissues of living organisms along the food chain.

Equally significant from the points of view of environmental fate and biological activity is the fact that solubilization of PAH in water occurs to an appreciable extent by the addition of

various surface-active agents, naturally occurring humic acid derivatives, proteins, purines and nucleic acids. As early as 1934, WINTERSTEIN and VETTER (37) showed the dissolving power of sodium deoxycholate by dissolving as much as 2g of B(a)P in 1 liter of 20% aqueous solution of this surfactant. Later EKWALL and SETALA (38) concluded that solubilization of B(a)P in water by sodium cholate, taurocholate, lauryl sulfate and other surface-active agents occurs through formation of association micelles between surfactant and PAH molecules. Partition coefficients of 28 PAH between aqueous monoethanolammonium deoxycholate and hexane were determined by DEMISCH and WRIGHT (39). Values found ranged from 5.8 to 24.4 which, when compared to the much higher octanol-water partition coefficients found by HANSCH, show the high water solubilizing power of the steroid surfactant used. It is interesting to note here that the most carcinogenic PAH showed the highest solubilities in the aqueous phase in these experiments.

SAHYUN (40) studied the solubilization of low molecular weight PAH and other aromatics in water by bovine serum albumin and showed that one molecule of albumin solubilizes one molecule each of fluorene and phenanthracene while about nine molecules of albumin are required to solubilize one molecule of anthracene. SAHYUN's experimental findings are in good correlation with his theoretical model, according to which solubilization by bovine serum albumin is due predominantly to hydrophobic bonding between PAH and protein. In this theoretical model, association between aromatic molecules and the solubilizing protein is an energetically favoured arrangement. Association reduces the surface of the

solute molecules exposed to the solvent to one half relative to the unbound dissolved state, bringing about a corresponding reduction in the energy of the hydrogen-bonded solvent cage, thereby stabilizing the dissolved PAH-protein complex. The energies of the hydrophobically bound state, E_b and of the original dissolved state E_s being proportional to the respective stabilities, they should be related according to the following formula:

$$\frac{dE_b}{dE_s} = \frac{d \log K}{d \log X} = \frac{1}{2}$$

where $\underline{K}$ is the number of PAH molecules bound per molecule of serum albumin and $\underline{X}$ is the solubility of PAH in the absence of the protein. Plotting log K against log X for a series of PAH and other aromatics, SAHYUN (40) obtained an approximately linear correlation, with a slope of 0.53, in good agreement with the above theoretical expression. FRANKE and BUCHNER (41) reached a similar conclusion regarding the mechanism of solubilization of pyrene and B(a)P by human serum albumin. Solubilization of PAH by purines and nucleic acids is accomplished by more complex mechanisms involving π-electron interactions and charge-transfer complexation. Solubilization of B(a)P in neutral aqueous media by various purine derivatives such as caffeine, theobromine theophylline and xanthine was first noted by BROCK and his collaborators (42) and was extended to other PAH by WEIL-MALHERBE (43). These results were later confirmed by BOYLAND and GREEN (44) who found that as much as 100 µM B(a)P and 1000 µM pyrene can be dissolved in a 0.1 molar aqueous caffeine solution. The fact that π-electron interactions are involved in the solubilizing

effect of purines is shown by changes in the ultraviolet absorption spectra of PAH and by the quenching of their characteristic fluorescence. Hydrogen bonding and hydrophobic bonding appear to be unimportant in this case since urea and lithium nitrate, which break hydrogen bonds easily have no effect on the solubilization of B(a)P by caffeine(44). BOYLAND and GREEN (45) have also studied the interaction of PAH with nucleic acids and found that a 0.05% aqueous solution of deoxyribonucleic acid (DNA) solubilizes 7 μM B(a)P and 22 μM pyrene. Similar findings were observed by LIQUORI and coworkers (46) for interactions of B(a)P and DB(a,h)A with DNA. The solubilization of PAH by DNA is also accompanied by shifts of the ultraviolet spectra and quenching of fluorescence, showing the involvement of π-electron interactions. However, in this case hydrophobic bonding and London dispersion forces also seem to play a significant role in stabilizing the dissolved species.

1.1.4 Electronic Structure and Indices:

As mentioned earlier, the molecular structure of PAH is characterized by the presence of multiple fused benzene rings. Carbon and hydrogen atoms, which constitute the basic molecular framework of such aromatic systems, usually all lie in the same molecular plane, each carbon atom contributing three of its valence electrons to form three stable σ-bonds with each of its neighbouring atoms. In addition, each carbon atom also contributes a fourth, highly mobile so-called π-electron to the molecular electron-pool. These electrons, instead of being localized in the field of any particular carbon atom, become extensively delocalized over the aromatic systems as a whole, and together form

a characteristic molecular π-electronic system. It is this π-electronic system which, to a large measure, determines the chemical and biological reactivity of any particular PAH compound. The energy levels of, and electron distribution within this system may be calculated in an approximate manner by the molecular orbital method (47-48). As a result, electronic indices, characterizing the electron distribution in various regions and around individual atoms of the molecule, are obtained in terms of molecular orbital coefficients. Some of these indices are:

a) the π-electronic charge density which, to a certain extent, characterizes the behaviour of individual atoms within the molecule towards electrophilic or nucleophilic reagents. In the case of unsubstituted PAH the value of this index is unity for all carbon atoms, since each of them contributed uniformly to the π-electron pool. However, substituion changes the electron distribution and corresponding changes appear in this index as well for different carbon atoms;

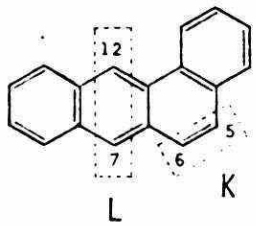
b) the bond order or mobile bond index, which is a measure of a particular bond's contribution to the total binding energy of the molecule. A high mobile bond index is indicative of a high probability of a π electron pair to be found in the region of that particular bond and consequently also indicates a relatively high reactivity of the particular bond in electrophilic addition reactions.

c) the free-valence number, which can be considered as a measure of noncoupled electrons around a particular atom and is particularly significant for free radical reactions but also has a certain importance in determining the

course of electrophilic and nucleophilic substitution reactions as well.

In the case of PAH compounds, the bond having the highest mobile bond index usually corresponds to the so-called mesophenanthrenic region, designated as the K-region, while the free valence numbers of the atoms in the so-called meso-anthracenic or L-region are indicative of the reactivity of that region.

The location of the K and L regions is shown below for BA:



The three electronic indices defined so far all refer to isolated molecules in their electronically most stable configuration, their ground electronic state, and are referred to as static electronic indices.

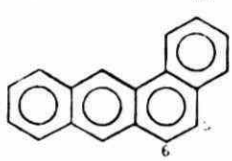
However, during the course of chemical reactions the neighboring, reacting molecules cause a perturbation in the most stable electronic configuration of the initial reactants. In order to establish a new chemical bond it was postulated that temporary localization of some electrons at appropriate regions of the reacting molecules is necessary (50). The lower the energy required to bring about this activated, hypothetical transition state, the faster the reaction occurs. This activation process results in the loss of some of the resonance energy of the initial molecule. The difference between the total π-electronic energies of the ground state and of the hypothetical transition state can be calculated by the above outlined molecular orbital method and is a measure of the activation energy required to bring about the particular chemical reaction.

Based on this concept, the PULLMANS (51) defined the following new set of dynamic electronic indices, the various localization energies:

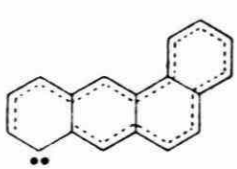
Carbon localization energy (CLE) is the energy needed to isolate a pair of π-electrons on one particular carbon atom;

Bond localization energy (BLE) is the energy needed to localize a pair of π-electrons between two adjacent carbon atoms;

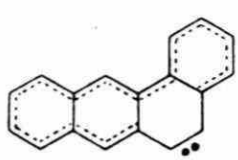
Para localization energy (PLE) is the energy needed to simultaneously isolate two π-electrons, one on each of two carbon atoms in para-position with respect to each other. Taking benz(a)anthracene as an example, the values of the various localization energies can be obtained (usually in β-units) by calculating the difference between the total π-electronic energy of the molecule in its ground state:



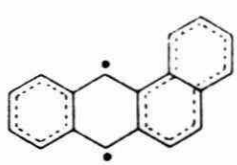
and that of the respective hypothetical activated transition states:



Carbon localization



Bond localization



Para localization

To better characterize the reactivity of the K- and L-regions of PAH, the PULLMANS (51) used the combinations of BLE + CLE_{min} for the K-region and PLE + CLE_{min} for the L-region, where CLE_{min} is the smaller of two CLE values calculated for the two carbon atoms involved.

TABLE V

SOME ELECTRONIC INDICES OF PAH

Compound Abbreviation	Bond	K-region		L-region
		Bond Order	BLE+CLE*$_{min}$ (in β units)	PLE+CLE*$_{min}$ (in β units)
A	1,2	1.738	3.53	5.38
Phen	9,10	1.775	3.36	-
P	4,5	1.777	3.33	-
B(e)P	4,5		3.37	-
B(a)P	4,5	1.787	3.23	-
BA	5,6	1.783	3.29	5.53
DB(a,h)A	5,6	1.778	3.30	5.69
DB(a,c)A	10,11	1.727	3.51	5.67
DB(a,j)A	5,6	1.780	3.31	5.66
Chry	5,6	1.754	3.38	-

* The Pullman's postulated electronic criteria for carcinogenic activity were: K-region BLE+CLE$_{min} \leq 3.31\ \beta$, and, if L-region is also present, PLE+CLE$_{min} \geq 5.66\ \beta$.

Some of these values for a number of PAH are shown in Table V, based on the PULMANS' review.

In addition to those mentioned so far, other useful electronic indices include the energy of the highest occupied molecular orbital (HOMO) and that of the lowest empty molecular orbital (LEMO), indicative respectively of the electron donor and acceptor properties of the particular molecule to which they refer and bear close relationship with the experimental parameters of ionization potential on one hand and electron affinity on the other.

1.1.5 Absorption and Emission Spectra of PAH

One of the most general physical characteristics of PAH is their ability to absorb electromagnetic radiation in the ultraviolet region (below 300 nm wavelength). Most PAH also absorb radiation in the near ultraviolet (300-400 nm) and some even in the visible (above 400 nm) regions. According to the laws of quantum mechanics, absorption of radiation can only occur in certain discrete quanta corresponding to the energy differences between the allowed energy levels of the particular atom or molecule. In the case of PAH, absorption of ultraviolet (or visible) light of well-defined wavelengths results in the promotion of π-electrons from their "ground state" molecular orbitals to certain "excited" π^*-orbitals at higher energy levels. The resulting electronic

transitions are called $\pi \rightarrow \pi^*$ transitions. The larger the difference between "ground state" and "excited state" energy levels, the higher is the energy or the shorter is the wavelength of the radiation to be absorbed to effect the particular promotion. $\pi \rightarrow \pi^*$ transitions to different energy levels result in the characteristic, highly structured ultraviolet absorption spectra of PAH.

While each spectrum is unique to individual PAH compared, a number of well known similarities and regular trends have been observed for the general class of PAH that are worth mentioning here. Thus, one can generally distinguish three groups of absorption bands, or band systems in the ultraviolet spectra of PAH. These are, according to the empirical classification by CLAR (9): the α-bands, which appear with low intensity (molar extinction coefficient $\varepsilon \approx 10^2\text{-}10^3$) at the long wavelength (low energy) and of the spectrum, the p-bands, which are more intense ($\varepsilon \approx 10^4$), usually appear at intermediate wavelenths and exhibit vibrational fine structure, and the β-bands, which appear with the highest intensity of the three ($\varepsilon \approx 10^5$) at shorter wavelengths corresponding to higher transition energies.

Linear annellation, that is elongation of the delocalized π-electron system in one dimension results in a regular shift of all three band systems towards longer wavelengths. The shift of the p-band usually occurs faster than that of the α and β-bands, particularly in the series of fully linear PAH compounds such as naphthalene-anthracene-naphthacene-pentacene, so that in the case of anthracene and naphthacene the weak

TABLE VI

ULTRAVIOLET SPECTRAL DATA OF SOME PAH

Compound Abbreviation	Solvent	α-bands λ(nm)	logε	p-bands λ(nm)	logε	β-bands λ(nm)	logε
A	Methanol/Ethanol			374.5	3.87	251.5	5.29
				354.5	3.86		
				338.0	3.75		
				323.0	3.47		
				308.0	3.15		
Phen	Methanol/Ethanol	345	3.46	292.5	4.30	251	4.78
		337	3.40	281	4.14	242	4.68
		329.5	2.52	273.5	4.18		
		322.5	2.54				
		314	2.48				
		308.5	2.40				
P	Methanol/Ethanol	371.5	2.40	333.5	4.71	272	4.67
		362	2.60	318	4.47	261.5	4.40
		356	2.71	305	4.06	251	4.04
		351.5	2.82	292	3.62		
B(e)P	Ethanol	366	2.80	331.5	4.52	289	4.72
		357	2.75	316.5	4.34	278	4.54
		348	2.90	304	4.12	267	4.46
						257	4.34
B(a)P	Ethanol	403	3.60	384.5	4.44	296.5	4.76
				363.5	4.36	284.3	4.66
				347	4.10	274	4.50
				330	3.76		
BA	Benzene (Ethanol at 275 nm)	385	3.04	359	3.76	290	5.10
				344	3.90	280	4.95
				329	3.90	267	4.67
				316	3.70	254	4.62
DB(a,h)A	Dioxane	394	3.01	350	4.16	299	5.20
		384	2.61	335	4.23	289	4.96
		374	3.00	322	4.28	280	4.63
B(k)F	Ethanol			400	4.16	308	4.75
				380	4.12	296	4.67
				361	3.86	282	4.42
						269	4.36

TABLE VI (cont'd)

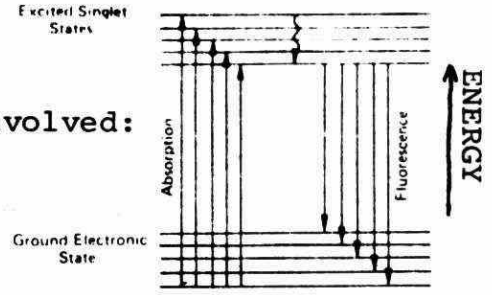
Compound Abbreviation	Solvent	α-bands λ(nm)	α-bands log ε	ρ-bands λ(nm)	ρ-bands log ε	β-bands λ(nm)	β-bands log ε
B(b)F	Ethanol			369	3.84	301	4.54
				350	3.98	293	4.36
				338	3.96	289	4.36
						276	4.36
Chry	Ethanol	360	3.00	319	4.19	267	5.20
		351	2.62	306	4.19	259	5.00
		343.5	2.88	295	4.13		
				283	4.14		
B(g,h,i)Per	Benzene (Ethanol at 289 nm)	406.5	2.70	387.5	4.50	303	4.76
				367	4.40	202	4.65
				348	4.00		
				331	3.82		
Cor	Benzene (Ethanol at 300 nm)	428	2.15	347.5	4.15	305	5.50
		420	2.12	341.5	4.85	2.90	5.10
		410	2.75	336	4.35	252	3.88
		402	2.65	325.5	4.45	228	4.08
		396.5	2.23	319.5	4.60		
		388	2.75	316.5	4.70		
		381.5	2.67				
		378	2.63				
		368.5	2.67				

α-band disappears under the stronger p-band. In the case of more angularly-shaped PAH the shift of the p-bands towards longer wavelengths is slower and as a consequence the disappearance of the α-bands occurs for higher members of the series.

U.V. spectral data for a few representative PAH are listed in Table VI , based on CLAR's (9) treatise.

In addition to ultraviolet and visible absorption spectra, fluorescence emission spectra are equally characteristic, useful and usually more sensitive for the identification and analysis of PAH. Fluorescence emission occurs whenever a molecule, in its first excited singlet state, loses its excitation or activation energy not by collisions or by chemical reaction but through radiative transition to the ground state. A thorough study of fluorescence involves the recording of two types of spectra namely the excitation and the emission spectra. Emission spectra are obtained by irradiating a compound at the wavelength of maximum absorption and detecting the emitted radiation by scanning at longer wavelengths . Excitation spectra, which should be very similar to absorption spectra, are produced by observing the change in intensity of the fluorescence at the wavelength of maximum emission while gradually changing the irradiating or excitation wavelengths. Fluorescence emission spectra of PAH compounds often appear as the mirror images of their absorption spectra, and are situated at longer wavelengths. This is easily understood by reference to the following schematic

representation of the energy levels involved:



where the heavy horizontal lines refer to the lowest vibrational energy levels of the ground and excited electronic states, the length of the vertical arrows being proportional to the energies (or inversely to the wavelengths) of the respective transitions. Examples of fluorescence excitation and emission spectra of some PAH are given in Table VII , using the data of PIERCE and KATZ (52).

TABLE VII

Fluorescence Excitation and Emission Spectra of Model PAHs in Hexane

Compound	Fluorescence excitation spectra, wavelength, nm	Fluorescence emission spectra, wavelength, nm
B(e)P	274, 284, 300, 309, 324, 329,349 358, 366	389, 397, 410, 421
B(a)P	267, 286, 297, 333, 347, 365,375 381	402, 407, 414, 424, 452
B(b)F	280, 293, 301, 340, 349, 368	404, 424, 446,
B(k)F	267, 294, 305, 323, 338, 350,357	402, 409, 418, 425,435,454
Per	365, 384, 407	438, 444, 465, 500
DB(cd,jk)P	263, 293, 302, 360, 377, 396,402	429, 436, 462, 466,488
B(gh,i)Per	288, 298, 329, 346, 362, 382	406, 415, 429, 438, 444
DB(a,i)P	285, 297, 316, 332, 354, 373,393	432, 432, 436, 447, 458,492
DB(a,h)P	299, 312, 378, 398, 421	451, 480, 514

[a]Most intense peak in each spectrum is underlined.

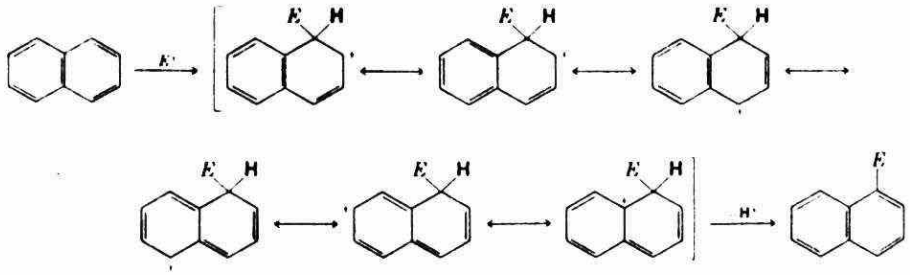
Phosphorescence emission occurs by radiative transition from the lowest excited triplet state to the ground state. (By definition, triplet state involves two decoupled electrons with parallel spins, while a singlet state is one where the resultant of all electronic spin projections is zero). Phosphorescence bands of PAH appear at even longer wavelengths and with lower intensity than fluorescence bands. Because of the extremely low intensity of the singlet-triplet transition, the excited triplet states of PAH are usually reached through radiationless deactivation of their excited singlet states.

Fluorescence and phosphorescence can also be distinguished by their respective radiative life-times, which are of the order of 10^{-7} sec for fluorescence and about 10^{-4} sec for phosphorescence. As an example, B(a)P exhibits phosphorescence emission maxima at 681.5, 697.5, 713.5, 745.5, 764, 783.5, 842. and 865 nm wavelengths (9).

1.2 Chemical Properties of PAH:

1.2.1 General: PAH compounds undergo all the characteristic reactions of aromatic systems, namely electrophilic substitutions, 1,2 - additions, 1,4 - additions, eliminations and radical reactions. The type of reaction that occurs depends of course on the nature of the particular PAH compound and the reagent. PAH are generally more reactive than simple benzene derivatives due to the possibility of stabilization of the reactive transition-state species by extensive delocalization of electrons (or resonance). This is illustrated, in the case of electrophilic substitution of naphthalene by the following reaction scheme where the various resonance structures of the intermediate

carbonium ion are shown in square brackets:



where E represents an electrophile (or electron-poor reagent)

The electronic properties of the previously mentioned K- and L- regions of PAH, having the highest mobile bond indices, free valence numbers and lowest ortho-and para-localization energies, determine the course of 1,2 - and 1,4 addition respectively. Reaction mechanisms involving resonance - stabilized free radicals are likely responsible for oxidation of PAH. Reactions of environmental importance of PAH were thoroughly reviewed by the U.S. National Academy of Sciences Committee on Biologic Effects of Atmospheric Pollutants (12). Interactions of PAH with biological systems in general and with proteins and DNA in particular were discussed in detail by the DAUDELS (10) and by ARCOS and ARGUS (11). A brief summary of these various types of reactions will be presented in the following paragraphs.

1.2.2 Substitution reactions:

Typical examples of electrophilic substitution processes include the reactions of PAH with nitrogen oxides, sulfur oxides as well as nitric and sulfuric acids. These reactions are likely to proceed through radical cation intermediates with NO_2^+ and SO_3^+ ions being the electrophilic reacting species, and result in various nitro derivatives or sulfinic and sulfonic acids. The most

active substituation sites in PAH are those in the L-region, particularly when located close to an active K-region, or, in cases without formal L-regions such as pyrene, at carbon atoms adjacent to ring fusions.

Facile nitration of B(a)P by nitric acid, resulting predominantly in the formation of the 6-nitro derivative, was reported by FIESER and HERSHBERG (53). Reaction of PAH with NO_2 is illustrated by the nitration of anthracene (54). Sulfonations of pyrene and B(a)P by sulfuric acid were reported by VOLLMAN and co-workers and by FIESER and HERSHBERG(53). SO_2 was also shown to react with PAH, particularly when the latter are present in the adsorbed state (55,56). Formation of a nitro-derivative of B(a)P on exposure to gaseous NO_2 at ppm levels was recently reported by PITTS and his collaborators (57). They observed mutagenic properties for this compound indicating its possible carcinogenicity. This contradicts the much earlier findings of WINDAUS and RENNHAK (58) according to which carcinogenic activity disappears in bromo-, nitro-, amino- and sulfonic acid substitution products of PAH.

These substitution products are generally more water soluble than the parent PAH, may significantly alter the biological activity of the latter and may escape detection in the benzene-soluble fraction of particulate matter. Furthermore, these substitution reactions may be of significant importance in polluted atmospheres, particularly at elevated temperatures encountered in combustion plumes and, for all these reasons, deserve much closer scrutiny

under real and simulated atmospheric conditions.

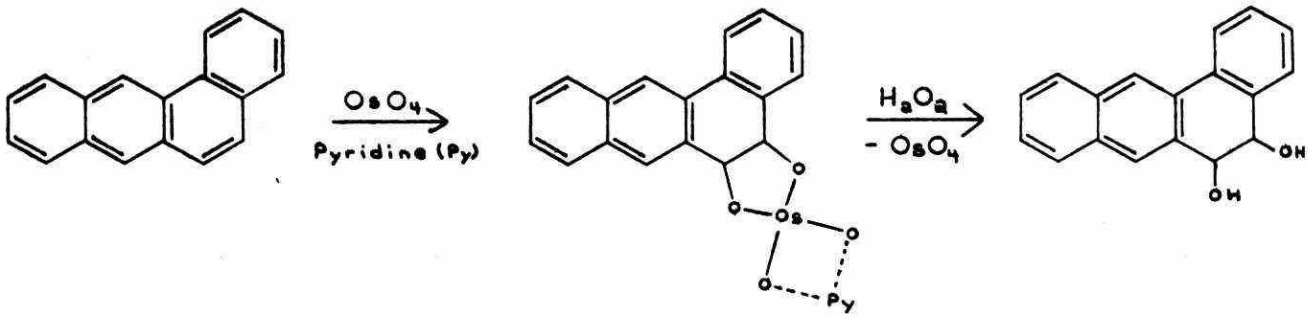
1.2.3 Addition reactions.

Addition reactions of PAH fall into two main categories: additions to a bond, or 1,2-additions and additions to two opposite atoms, or 1,4-additions. In classical organic chemistry osmium tetroxide and ozone are known to be some of the best bond addition reagents. In PAH compounds, some bonds that are particularly reactive towards these bond reagents correspond to the earlier mentioned K-regions. Reactivities of particular PAH compounds towards addition of OsO_4 correlates well with their smallest bond (or ortho-) localization energies, and increasing tendency to add OsO_4 coincides with increasing carcinogenic power as well, as shown in the following Table (10):

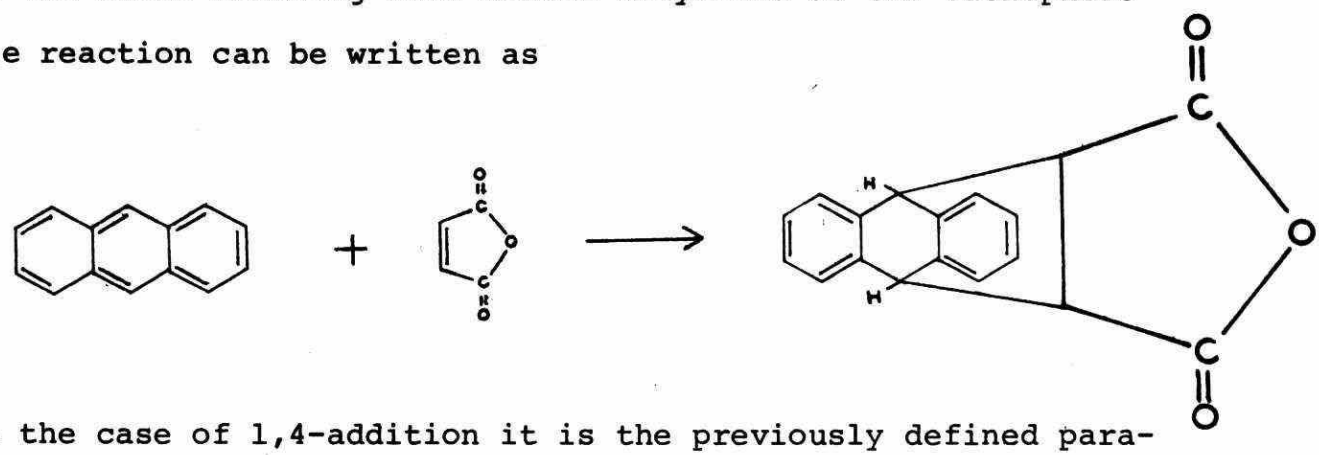
TABLE VIII

Compound	Relative rates of O_sO_4 addition	Smallest ortho-localization energy (in B units)	Carcinogenic Activity
Benzene	0	1.53	-
Phenanthrene	0.2	1.06	-
BA	1	1.03	+
DB(a,h)A	1	1.04	++
B(a)P	2	1.03	+++

It is thought that this type of reaction may play an important role in the fixation of carcinogenic PAH to biological subtrates.
Addition of OsO_4 to a double bond eventually results in its oxidation to a vicinal diol, illustrated, in the case of BA, as follows:

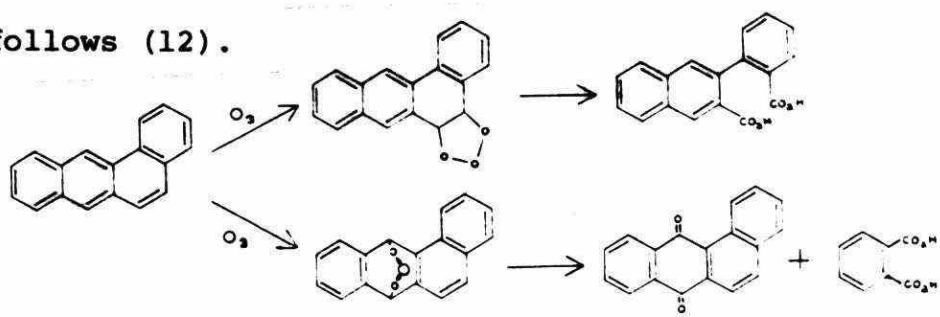


The bestknown example of 1,4-additions in the so-called Diels-Alder reaction (or diene synthesis). In the case of anthracene as the diene reacting with maleic anhydride as the dienophile the reaction can be written as

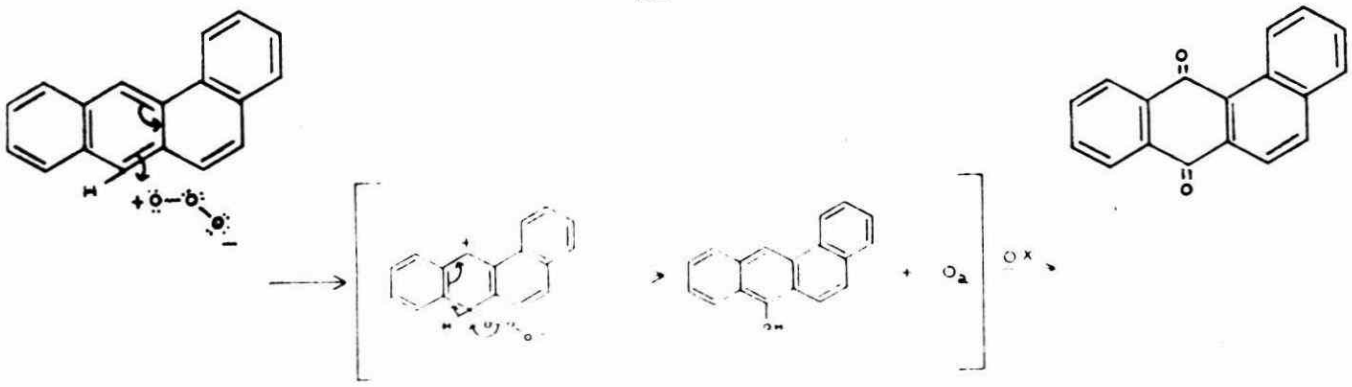


In the case of 1,4-addition it is the previously defined para-localization energy which determines the reactivity of PAH compounds. As the PLE decreases in the series of linear polyacenes with increasing number of condensed rings, so does their reactivity towards maleic anhydride increase.

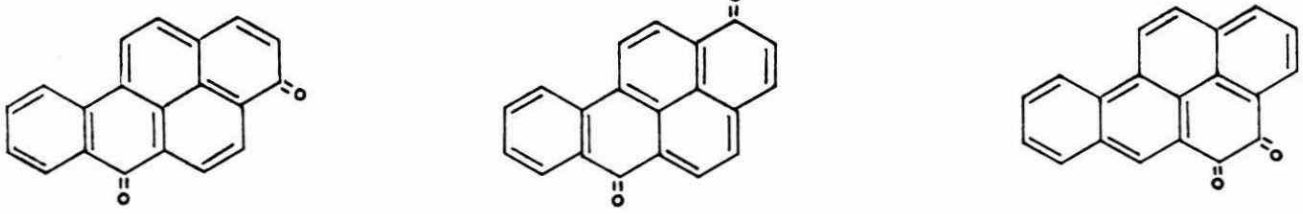
1.2.4 The reactions of PAH with ozone may occur both by 1,2-addition and 1,4-addition, which is illustrated in the case of BA as follows (12).



Reactions of PAH with ozone may also proceed through a different mechanism, namely electrophilic substition, ozone acting as the electrophile (12):



A similar mechanism is probably operating in the case of oxidation of B(a)P by ozone, resulting in a 3:1 mixture of the corresponding 3,6- and 1,6-quinones and a trace of the 4,5-dione (58):



Reactivities of several PAH towards ozone in water were measured by IL'NITSKII et al (59) and the corresponding approximate rate constants and half-lives were calculated by RADDING et al (16). The estimated half-lives ranged from less than 10 minutes for dimethylbenzanthracene, to about 1 hour for B(a)P. More recently LANE and KATZ (60) investigated the reactivity of thin layers of B(a)P, B(b)F and B(k)F towards ozone. While the half-life of B(a)P agreed well with the above-mentioned value, they found B(b)F and B(k)F much less reactive.

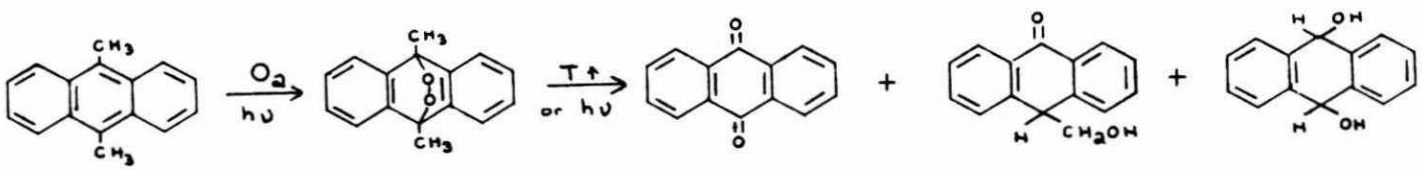
1.2.5 Photooxidation of PAH

As mentioned earlier, most PAH containing three or more benzenoid rings are capable of absorbing solar radiation above 300 nm wavelengths, resulting in excited electronic states. In the presence of light and oxygen PAH undergo photooxidation.

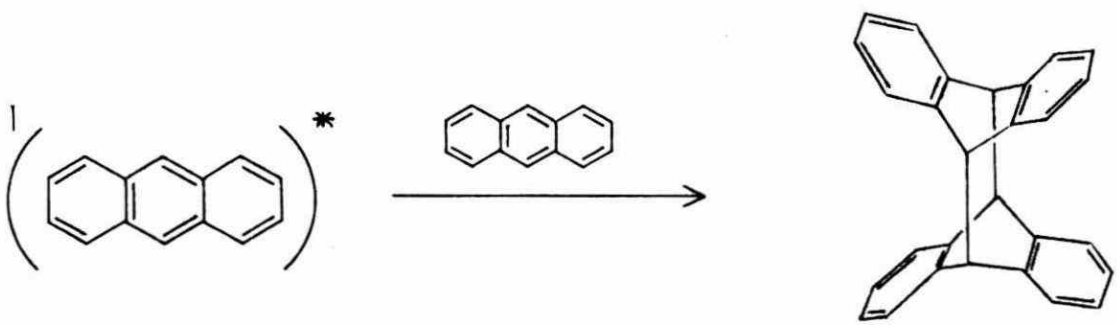
at varying rates depending on the temperature, their physical state, as well as their individual structure. Photooxidation of PAH involves energy transfer from their excited states, mainly from their triplet states (12, 16, 61) to produce excited, singlet oxygen molecules in deactivating collisions with ground-state oxygen. Singlet oxygen then reacts with PAH in their ground state to form oxidized products. The process can be written schematically as follows (12):

$$M \xrightarrow{h\nu} {}^1M^* \xrightarrow{{}^3O_2} {}^3M^* \xrightarrow{{}^3O_2} {}^1O_2{}^* + M \longrightarrow MO_2$$

where M, ${}^1M^*$ and ${}^3M^*$ denote PAH in their ground state and their singlet and triplet excited states respectively. The initial reaction products, whenever the so-called meso-anthracenic positions are available, are usually endo-peroxides, which can be converted into a variety of products including mainly quinones, on heating or further photolysis, e.g. (12):



As an alternative to reaction with oxygen, a PAH molecule in its excited singlet state may react with another molecule of PAH in its ground state to form a photodimer, e.g:



While the aforementioned reactions were mainly studied in organic solutions, their basic mechanism is expected to apply under environmental conditions as well. Indications are (12) that reactivity of PAH is greatly enhanced in the adsorbed state. Investigations by several groups of researchers (63,64,60,23) indicate that photochemical modification of most PAH compounds occurs within a matter of a few hours or at most a few days under environmental conditions. While the role of the singlet oxygen in photooxidation of aqueous PAH solutions has recently been discounted by ZEPP and SCHLOTZHAUER (444) largely on theoretical grounds, KATZ (446) and TOSINE (447) on the other hand have experimentally demonstrated its importance. In any case, the generation of this highly reactive species by photoexcited PAH has been amply demonstrated (61) and may in fact prove to be one of the most significant consequences of PAH photochemistry.

1.2.6. Miscellaneous Oxidation Reactions of PAH:

Oxidation of PAH with various one-electron metal salt oxidants, such as potassium ferricyanide, ceric sulfate, manganese dioxide, etc., occurs relatively easily, through removal of one electron from the polycyclic system, resulting in the formation of radical cations as the primary products. These unstable intermediates then react further with available nucleophiles (including water and unoxidized PAH) or with oxygen, to form endoperoxides, diols, quinones, aldehydes and dimers. Adsorption on active surfaces, such as alumina, silica or soot, can also produce radical cations directly, and so can irradiation and treatment with strong Lewis acids (12). As examples, the one-electron transfer oxidation of 7,12-dimethylbenz(a)anthracene was described by FRIED and SCHUMM (65) and that of B(a)P was investigated by WILK and

collaborators (66). All the various reaction products identified could be accounted for by the generation of radical cations followed by nucleophilic attack of the solvent or of the unoxidized PAH at the most active sites. Reactions of PAH radical cations with electron donors usually regenerate the PAH at least partly in an excited state, which in turn might lead to the generation of singlet oxygen in biological systems, in the absence of light (12), possibly resulting in genetic damage. Other oxidants of environmental importance, such as hydroxy, hydroperoxy, alkyl-and arylperoxy-radicals, and peroxides, all react relatively readily with PAH (12, 16). For example B(a)P reacts with benzoylperoxide to yield the 6-benzoyloxy derivative (68). Peracids, such as peracetic acid (CH_3CO_3H) are rather powerful oxidants of PAH and have a tendency to induce formation of epoxides (67), and eventually result in acidic decomposition products of PAH. Radical cation and epoxy-derivatives of PAH can be formed in biological systems through electron-transfer and enzymatic mechanisms. The importance of biological oxidations in activation of PAH will be discussed briefly in the following paragraph.

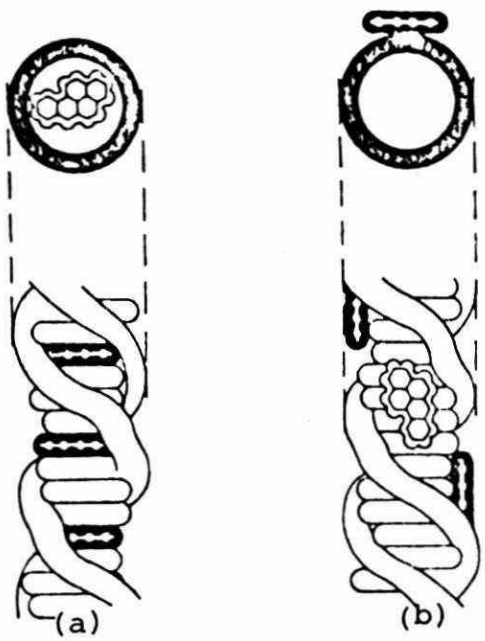
1.2.7 Interactions of PAH with proteins and nucleic acids.

In order to explain the carcinogenic activity of PAH compounds, scientific attention has been focussed since the 1940's on their interactions with essential cellular macromolecules: the proteins and nucleic acids. Historically, before the discovery by

WATSON and CRICK (69) of the role of DNA as depository of genetic information, proteins were thought to play the determining role in the course of carcinogenesis and the possibility of a direct interaction between a carcinogen and a protein was first suggested by SCHMIDT in 1941 (70). Non-covalent interactions of PAH with proteins and nucleic acids were already mentioned earlier in this report (para. 1.1.3) in connection with their solubilization. In the case of proteins, it was shown (40) that non-covalent interactions with PAH are mainly of hydrophobic nature. The possibility of charge-transfer interactions was also suggested, in which electrons are transferred either from the highest occupied orbitals of the hydrocarbons to the lowest unoccupied triplet levels of the proteins (72). In either case, semiconduction would be induced in the proteins, bringing about the following type of keto-enol tautomeric rearrangement at critical hydrogen-bonded peptide linkages:

However, this attractive hypothesis has never been satisfactorily demonstrated at the experimental level and has also been severely criticized on theoretical grounds (73). As for non-covalent interactions of PAH with nucleic acids, we have also mentioned their in vitro occurrence in solubilization phenomena and the whole field was thoroughly reviewed by ARCOS and ARGUS (11).

Briefly, solubilization of PAH by DNA can be accounted for by two types of non-covalent binding mechanisms: (a) "internal" binding or "intercalation" of PAH compounds between two planar purine-pyrimidine base pairs, the basic constituents of the well-known DNA double helix structure; (b) "external" binding of PAH to DNA, perpendicular to the planes of the purine bases. These two possibilities are shown schematically in Fig. 1:



Models for Non-Covalent PAH-DNA Interactions

The intercalation type of binding was first suggested by BOYLAND and GREEN (45) who pointed out that planarity, steric fit and geometric similarity between the particular PAH compound and a simple purine-pyrimidine base pair are important requirements for this type of interaction. Definitive proof of BOYLAND's intercalation hypothesis was provided by NAGATA and collaborators (74)

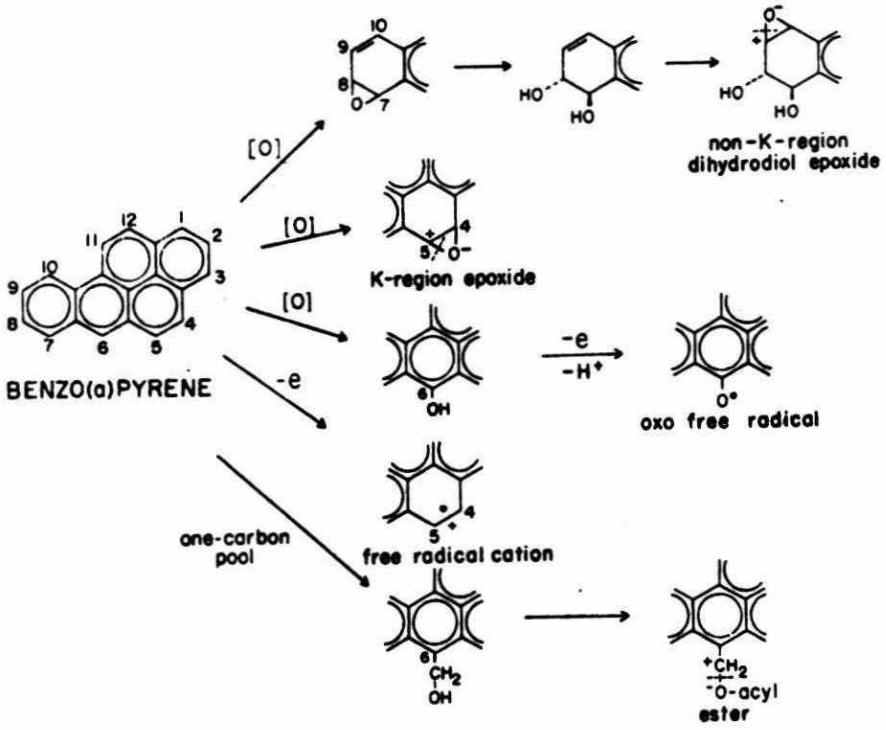
who experimentally determined the spatial orientation of PAH compounds associated with DNA molecules, using the elegant method of differential flow dichroism in which light polarized in a plane parellel or perpendicular to the flow of PAH-DNA complex was absorbed to different extents depending on the specific orientation of the chromophore relative to the macromolecule. In this way NAGATA et al. (74) were able to show that phenanthrene, pyrene and B(a)P were interacting with DNA by intercalation, whereas coronene, naphthacene and pentacene interacted by external binding.

Whatever the significance of the above-mentioned non-covalent interactions of PAH with proteins and nucleic acids, the balance of evidence points to the fact that there is no significant correlation between the extent of these types of interactions and the carcinogenic power of individual PAH compounds.

Covalent binding of PAH with cellular macromolecules is therefore more likely to be the causative factor in their carcinogenic power and, as a matter of fact, covalent binding of almost every known chemical carcinogen or some of their derivatives to DNA, RNA and proteins was observed (12). Covalent binding of PAH to proteins was first demonstrated experimentally by MILLER (75) who, using fluorescent techniques found that B(a)P was strongly bound in-vivo to mouse skin proteins. Covalent binding of PAH to mouse skin proteins was then extensively investigated by HEIDELBERGER and his associates (76, 77, 78). While the precise nature of the

of the covalent binding could not be definitely established on the molecular level, these authors, using radioactive tracer techniques were nevertheless able to show excellent correlation between the carcinogenic power of some PAH compounds and their ability to bind to a specific soluble protein.
HEIDELBERGER and collaborators (79) were subsequently successful in purifying this same protein.
Covalent binding of PAH to mouse skin DNA was also conclusively demonstrated in-vivo by several authors (80, 81, 82) and the amount of binding was found to be proportional to the carcinogenic activity of individual PAH compounds.
It must be stressed that all the above-mentioned covalent interactions have only been observed in-vivo, and no covalent binding is known to occur between any PAH compound per se and DNA, RNA or proteins in the test tube. Consequently, PAH must be chemically modified in living organisms to a reactive derivative prior to the formation of a covalent bond between it and the macromolecule. Such chemical modifications can be brought about in-vitro by treatment of PAH-DNA mixture, with ultraviolet or x-ray irradiation (83, 84), with hydrogen peroxide (85) or with iodine (85, 86). In biological systems, that is in-vivo, chemical modification of PAH to reactive species is brought about through metabolic activation. This was demonstrated experimentally by GELBOIN (87) and by GROVER and SIMS (88) by incubating PAH with rat liver homogenates, adding DNA and observing formation of covalent bond between PAH and DNA. By analogy with the in-vitro data, activation

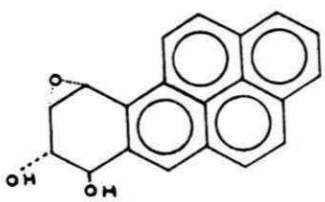
of PAH <u>in-vivo</u> was postulated and later found to be of oxidative nature. As to the exact chemical structure of the activated PAH species, several probabilities are known to exist, as shown in the case of B(a)P, in Figure 2(89):



Schematic Representation of Possible Active Metabolites of B(a)P

All these electrophiles have been proposed as reactive intermediates and metabolic formation and covalent binding with DNA and proteins was later demonstrated experimentally for most of them. Thus, the metabolic formation of K-region epoxides was first shown by SELKIRK et al. (90) in the case of DB(a,h)A and by GROVER et al. (91, 92) in the case of B(a)P. It was also shown (93, 94) that covalent

binding of these epoxides with DNA occurs to a much greater extent than in the case of the parent hydrocarbons. However, interest has recently switched away from the K-region epoxides due, on one hand, to their limited or no carcinogenic activity (95) and, on the other hand, to the difference in products obtained when their reactions with DNA were compared to those between the parent hydrocarbons and DNA in cellular systems (96, 97, 98). The existence of the 6-phenoxy free radical formed by biological oxidation of 6-hydroxybenzo(a)pyrene was demonstrated by NAGATA and his collaborators (99, 100) but again the 6-hydroxy derivative itself does not exhibit significant carcinogenic activity. The existence of radical cations as active metabolic intermediates of PAH was suggested and investigated by CAVALIERI and co-workers (101, 102), while side chain oxidation and hydroxymethylation was favored as activation mechanisms by others, (103, 104, 105, 106). However, the weight of currently available evidence is overwhelming in favor of non-K-region dihydrodiol epoxides as the most active metabolites for covalent binding of PAH to DNA (97, 107, 108) and current research is focussed on clarifying the molecular structure of covalently bound PAH-DNA products. As an example, MEEHAN, STRAUB, and CALVIN (109) were able to show that specific covalent binding of the non-K-region diol epoxide of B(a)P:

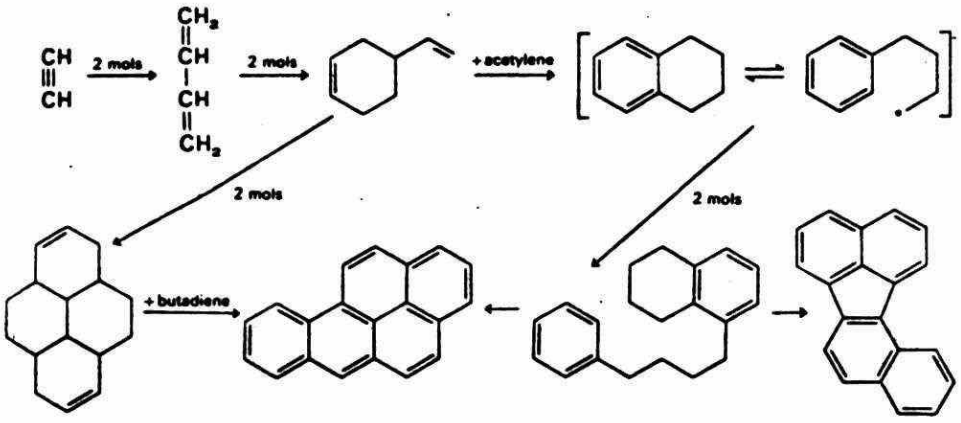


occurs to the deoxyguanosine and deoxyadenosine bases in DNA. Such detailed structural investigations are of utmost importance for full understanding of the mechanism of action by which some PAH may induce cancer.

2. FORMATION AND SOURCES OF PAH IN THE ENVIRONMENT:

2.1 Formation -

It is at present generally recognized that formation of PAH occurs during incomplete combustion of almost any kind of organic matter, including coal, oil, wood, natural gas, various animal and vegetal lipids, carbohydrates, proteins, amino acids, etc. Although the mechanism of their formation is rather complex and depends on a number of factors, including temperature, nature of the combustible material, time, and availability of oxidizing or reducing atmosphere, the nature of the overall reaction is fairly well established. "Cracking" of carbon compounds at high temperatures, repolymerization of the resulting molecular fragments and aromatization of the polycyclic carbon skeletons by loss of hydrogen has been known and practiced in petroleum processing for a number of years. Aliphatic carbon-carbon bonds and carbon-hydrogen bonds break down relatively readily at pyrolysis temperatures (500-800^{o}C), yielding molecular fragments of free radical character which then undergo recombinations. At the temperatures of pyrolysis, and in chemically reducing atmospheres prevailing at the center of flames, aromatic ring systems are the most stable ones among the various molecular species present and their relative stability leads to the gradual accumulation of condensed aromatic structures of increasing complexity as the pyrolysis and pyrosynthesis progresses in time during the combustion process. The following pyrosynthetic pathways leading to the formation of B(a)P and B(j)F, were proposed by Badger and his co-workers,[110-112] based on an extensive series of pyrolysis experiments involving simple, C^{14}-labelled hydrocarbons such as acetylene, butadiene, ethylbenzene, styrene, n-butylbenzene, 1-phenylbutadiene, and tetralin:



Similar routes may lead to the formation of most of the known PAH produced in combustion processes. While formation of acetylene and ethylene was actually observed during the thermal decomposition of many organic materials, complete breakdown of the starting material to two-carbon radicals is not necessary for the pyrosynthetic formation of B(a)P and of other PAH compounds. As a matter of fact, the availability of higher molecular weight fragments necessary for pyrosynthesis of B(a)P increases the yield of this and of other compounds in combustion processes. Thus, the use of n-butylbenzene as the starting material was shown to result in the highest yield of B(a)P and B(j)F, as per the above reaction sequence.[112] The relative importance of C_4-units, such as butadiene, in the formation of B(a)P was further demonstrated in recent comparative pyrolysis experiments with simple amino

acids, where the highest yield of B(a)P was obtained from proline, the only amino acid among the ones examined which produced butadiene on pyrolysis.[113] Badger and collaborators also examined the temperature dependence of pyrolytic PAH formation, using n-butylbenzene as the starting material. They showed that for most PAH there is an optimum temperature range of approximately 650°C to 850°C, beyond which the yield decreases rapidly.[111]

In order to explain the occurrence of PAH in fossil fuels in seemingly unpolluted soils and in marine sediments it has been suggested that PAH may also be formed biosynthetically by plants and lower organisms,[114-117] and by low-temperature processes similar to those which bring about the transformation of plant and animal matter into peat and lignite. However, while the possibility of PAH formation through such processes can not be discounted, they remain largely either unconfirmed or highly controversial. Thus, the biosynthetic formation of PAH by plants, formerly thought to have been conclusively demonstrated, is now largely discredited by the negative results found with plants grown in absolutely unpolluted media.[118, 119] It has also been suggested that PAH formed in natural combustion processes, such as forest fires, are structurally distinct from those produced in anthropogenic sources.[120] This suggestion, based on the observation of different PAH homolog distributions in recent marine sediments as compared to those in airborne particulate matter, has also been vigorously disputed and the difference in homolog distribution explained on the basis of partitioning effects due to differences in water solubility.[121]

2.2 Sources, and Emission Data:

2.2.1 Identification of Sources:

As mentioned in the previous section, almost any combustion process involving carbon- and hydrogen- containing compounds may be a source of PAH emission to the environment. The amount of PAH formed depends primarily on the efficiency of the combustion process itself, highly efficient combustion resulting in essentially complete conversion of carbon and hydrogen to carbon dioxide and water, and inefficient burning in oxygen-poor atmospheres resulting in the formation of relatively high levels of PAH. The amount of PAH actually emitted also depends of course on the efficiency of the pollution control equipment used, if any.

Sources of PAH include natural as well as man-made ones. Natural sources such as forest fires and volcanic eruptions have contributed to man's exposure to PAH since prehistoric times. Other natural sources of PAH may possibly include biosynthesis by lower organisms and low-temperature natural processes involving decaying organic matter. Anthropogenic sources of PAH may be further classified into industrial and non-industrial ones. Among the latter types, cigarette smoking is the single, most significant source of direct, personal exposure to carcinogenic PAH. Other non-industrial anthropogenic sources of PAH include the home cooking, frying, smoking, roasting and charbroiling of foods, agricultural burning, home heating, and some leisure-type activities involving the use of internal combustion engines such as outboard motors and gasoline-powered lawn mowers.

Industrial sources are usually subdivided into mobile and stationary ones, the latter including heat - and power - generating facilities, coke production, asphalt production, petroleum catalytic cracking, iron and steel sintering, aluminum reduction, carbon black manufacturing, as well as municipal and commercial incinerators.

2.2.2. Emission Estimates

While qualitative identification of actual and potential sources of PAH is a relatively simple undertaking, development of quantitative estimates of emission factors is a much more difficult task. The following are some of the causes for such a difficulty:

a) scarcity of reported emission data;

b) even the few reported emission estimates are usually based on a limited number of tests, involving few, if any, duplicate runs for any particular type of source. Thus, the representativeness of the results may be questionable;

c) emission estimates may vary over several orders of magnitude for any particular source type, depending on individual operating parameters, age and condition of production equipment, efficiency of control equipment and variability of feed composition. Thus, the reliability and general applicability of the results may be questionable.

d) emission estimates may be based on different sampling and analytical methods, resulting in highly questionable comparability of different studies involving the same type of emission source;

e) there is also a significant degree of uncertainty with regard to the contribution of emissions from other sources, particularly when emission estimates are based on atmospheric sampling in the vicinity of sources.

f) While it is known that a great number of various PAH compounds may be formed in any source, only a few, at most, are usually measured in any particular study, due mainly to cost considerations and time constraints, let alone analytical difficulties. Since B(a)P is the most frequently measured PAH compound and its biological effects are also well known, the amount of B(a)P has traditionally been considered as an indicator of total PAH present and emission factors are usually expressed in terms of B(a)P units. However, despite numerous attempts to find a definite correlation between the amount of B(a)P and various other PAH compounds, no such correlation has been conclusively demonstrated and consequently the amount of B(a)P may not be a good indicator of the nature, composition or amount of total PAH formed. For the same reason, B(a)P may not even be the best indicator of potential health hazards either, due to the possible presence of cocarcinogenic and/or anticarcinogenic PAH compounds, to be discussed later in Chapter IV.

In spite of these various difficulties and uncertainties a number of emission estimates were developed for various PAH source types. The most comprehensive data base was developed in 1967 by HANGEBRAUCK[122] and collaborators and became the standard reference document for most subsequent workers in the field. This document has been very substantially updated and enlarged under the sponsorship of the U.S. Environmental Protection Agency and made available very recently, for review, in draft form.[123] We have used the emission factors developed in this latter document to calculate estimated quantities of B(a)P emissions from various known and potential PAH sources in Ontario, based on available production or fuel consumption data. The results are presented in Table IX.

While these estimates have to be given with some reservations, in view of the difficulties mentioned above, they are probably valid within an order of magnitude and can no doubt serve to indicate the relative importance of the different source categories as contributors of environmental PAH pollution in Ontario. The estimated B(a)P emission factors given in Table IX refer to the "intermediate" or "best" estimates given in the abovementioned EPA document, unless indicated otherwise.

A preliminary survey of a few potential PAH emission sources in Ontario[124] provide some additional data, particularly with regard to graphite electrode manufacturing process emissions. Other preliminary data given in the same draft report, whenever comparable, are in reasonable agreement with estimates given in Table IX. Some brief comments on individual source categories and available control equipment follows.

TABLE IX

Some Sources and Potential B(a)P Emission Estimates in Ontario

SOURCE	ANNUAL PRODUCTION OR FUEL CONSUMPTION IN ONTARIO	YEAR	ESTIMATED B(a)P EMISSION FACTOR (123)	ESTIMATED ANNUAL B(a)P EMISSION IN ONTARIO (kg)	REMARKS	REFERENCE
Heat and Power Generation:						
Coal Fired -						
Power Plants	7.68×10^9 kg	1976	1.6 µg/kg	12.30	A	125
Industrial Boilers	5.14×10^8 kg	1976	0.93 µg/kg	0.48	B	125
Oil Fired -						
Industrial Boilers	3.48×10^9 kg	1976	1.1 µg/kg	3.83	S	126
Commercial/Institutional Boilers	1.06×10^9 kg	1976	40 µg/kg	42.52	S	126
Residential Furnaces	3.86×10^9 kg	1976	2.2 µg/kg	8.50	F	126
Gas Fired -						
Industrial Boilers	9.23×10^9 m^3	1976	0.74 µg/m^3	6.8	G	127
Commercial/Institutional Boilers	4.33×10^9 m^3	1976	7.6 µg/m^3	32.93	H	127
Residential Furnaces	3.85×10^9 m^3	1976	2.0 µg/m^3	7.70	I	127
Heat & Power Generation Total:				115.06		
Coke Production	4.36×10^9 kg	1977	2.14 mg/kg	9331.5	J	128
Asphalt, Hot Road Mix Prod'n	4.46×10^9 kg	1976	0.06 µg/kg	0.026	K	129
Forest Fires	5.44×10^9 m^2	1976	1.5 mg/m^2	8,160	M	130
Mobile Sources						
Gasoline-powered vehicles except Motorcycles	11.9×10^9 L	1976	16 ug/L	190.4	N	131
Motorcycles(2-and4-cycle) and snowmobiles	8.0×10^7 L	1976	1.6 mg/L	128.0	Q	131
Diesel-powered vehicles (mostly trucks)	1.43×10^9 L	1976	3.7 ug/L	5.29	O	131
TOTAL FROM ALL OF THE ABOVE SOURCES				17,930.28 kg		

REMARKS TO TABLE IX

A Figures quoted are for Industrial consumption by Electric Utilities

B Figures quoted are for "Industrial Consumption" excluding consumption by Electric Utilities

F Figures quoted are the net sales for "Residential, apartment and farm use".

G Figures quoted represent "industrial demand" and may include more than just consumption by boilers; in addition figures were converted to millions of m^3 from millions of ft^3.

H Figures quoted represent "commercial demand" and may include more than just consumption by boilers; in addition figures were converted to millions of m^3 from millions of ft^3.

I Figures quoted represent "Domestic and farm demand" and may include more than just consumption by residential furnaces; in addition figures were converted to millions of m^3 from millions of ft^3.

J Ontario's figure estimated on the basis of 90% of Canadian production located in the province as per statistics of previous years.

K Figures given are for amount of asphalt sold for paving purposes.

M Reference (130) gave a value in acres which was then converted to $(metres)^2$ by conversion: acre=43560 ft^2=4046.86m^2

N Figures given reflect net sales of gasoline which no doubt includes consumption by automobiles as well as motorcycles.

O Figures given reflect net sales of diesel oil which no doubt includes consumption by both automobiles and trucks

Q Calculation based on estimated fuel consumption.

S Total oil consumption (both light and heavy) was found by using a density of 7.210 lb/U.S. gal (864.7249 Kg/m^3) for light oil and 7.830 lb/U.S. gal (939 .0841 Kg/m^3) for heavy oil.

2.3 Individual Source Categories and Control Equipment:

2.3.1. Forest Fires and Other Uncontrolled Burning of Organic Matter:

As indicated in Table IX, forest fires in Ontario are probably among the most significant sources of PAH emissions on the global scale. The estimated emission factors are based on extrapolation from laboratory experiments[132] and, due to the variable nature of combustible material and of the combustion process under different geological seasonal and weather conditions, no "best" estimate is available. However, the figures do provide a good indication that PAH emissions from forest fires are of the same order of magnitude as those from the most significant industrial sources. While direct population exposure and consequently health hazard are much less significant, nevertheless the contribution of forest fire emissions to the global environmental PAH burden is by no means negligible. This points to the importance of prevention and extinction as the only feasible means of control.

Although provincewide emission levels from other uncontrolled burning of organic matter, (such as accidental fires, intentional burning of grass clippings, leaves, branches, as well as wood burning in residential fireplaces and charcoal combustion in backyard barbecues) could not be estimated in this study due to unavailability of data on materials consumed, the few published emission factors (e.g., 4.2 μg B(a)P/m^3 in smoke from open burning of grass)[122] and the example of forest fire emissions indicate that such sources may contribute to a significant degree

to PAH pollution. The problem in these cases is compounded by the uncontrolled nature of emissions and direct exposure of relatively large populations, this pointing to the need of stepped-up monitoring and regulatory activity in this area.

2.3.2. Cigarette Smoke:

A number of carcinogenic PAH, including B(a)P, DB(a,h)A, DB(a,i)Pyr, B(b)F and B(j)F, as well as several phenolic tumor promoters and other tumorigenic agents (e.g., 2-Naphthylamine) were identified and quantified in cigarette smoke, which has been positively linked with an increased risk of developing lung cancer.[133] Although the contribution of cigarette smoke to the total environmental burden of PAH may seem rather small in Ontario (less than 1 kg of B(a)P, based on recent population and cigarette consumption statistics[134] and published B(a)P content in the particulate phase[135]) compared to other sources, it is nevertheless the single most significant source of PAH in terms of personal exposure and impact. Thus, a one-pack-a-day smoker may inhale about 1 µg of B(a)P per day. This quantity is more than fifty times as much as the one inhaled in a day by the average non-smoker living in an average North American "coke oven city" having an atmospheric B(a)P concentration of 1.2 ng/m^3. A frequently overlooked aspect of the problem of tobacco smoking is the hazard it represents to the non-smoking population exposed to it. It has been shown[136] that B(a)P concentration in side-stream cigarette smoke is significantly higher than in the main-stream itself. Moreover, the particulate portion of cigarette smoke, with a reported mass median aerodynamic diameter of about 0.4 µm, is al-

most entirely within the respirable size range. While inhalation of PAH from the main-stream smoke may be controlled to a certain extent by the use of filters, no such control is possible for side-stream smoke, which may result in PAH pollution levels, particularly in enclosed areas, exceeding by one or two orders of magnitude[137] those found in normal ambient atmospheres.

2.3.3. Coke Production:

Coke is manufactured by high-temperature carbonization of bituminous coal in slot-type coke ovens using the so-called by-product process in which benzene and coke-oven gas are recovered in the by-product plant. The process is an integral part in the fabrication of steel and, as such, is of major economic importance. However it is also the single largest industrial emitter of PAH and of a large number of other toxic chemicals including 2-Naphthylamine, arsenic, beryllium, selenium, benzene, pyridine, ammonia, hydrogen cyanide etc., which together constitute the complex mixture of coke oven emissions.[138] As far as PAH are concerned, about sixty of these compounds were detected by LAO and co-workers[139] in coke-oven emissions, some of the major constituents being phenanthrene, anthracene and their methyl- and ethyl-derivatives, fluoranthene, pyrene and their methyl-derivatives, BA, Chr, Me-Chr, B(k)F, B(b)F and B(a)P.

The coke production process involves a series of operations, namely charging, coking, pushing, quenching and by-product recovery. As slot-type coke-ovens are normally equipped with a chemical recovery system, most of the PAH emissions occur through leakage during any of the above operational phases.

a) Charging emissions: these result from volatilization of coal as it is being charged into the red-hot oven. They are fugitive in nature and usually escape through charging ports and hoppers. Control of emissions may involve the use of stage charging, or sequential charging techniques, or the installation of scrubber systems mounted on larry cars. The U.S. EPA has recently identified stage charging as best control technology for charging operations.[140] This technique is based on charging coal from one, or a maximum of two larry car hoppers at a time into the red-hot ovens, while maintaining the ovens under a slight negative pressure by use of aspiration in the standpipes thereby drawing emissions into the battery main.

b) Emissions during coking may occur through topside leaks and door leaks. These are also fugitive emissions. Sources of topside leaks include oven lids covering the charging ports, standpipe caps, and various cracks. These emissions can only be controlled by frequent inspections and careful sealing or "luting". Door leaks may occur through cracks and inadequately sealed doors on both ends of each oven. Control of these emissions require proper maintenance, use of doors specially designed to provide tight closure and individual hoods over each door.

c) The pushing operation - discharging of the incandescent coke from the oven into a railcar - is another major potential source of PAH emissions if not controlled properly.

Again, the emissions are fugitive in nature, carried upwards by the movement of hot air masses in contact with the hot coke. Control of emissions can be achieved by installation of a shed over the coke side of the battery and use of control devices such as Venturi scrubbers and wet electrostatic precipitators. Control efficiency of up to 90 per cent can be achieved in this manner, as experience in Hamilton coke sheds has shown.

d) Quenching emissions occur when the hot coke is being doused with a large volume of water in the quench tower. These emissions at present are largely uncontrolled and the amount emitted is largely dependent upon the design of the tower and the type of baffles used. The use of clear water is thought to provide some degree of control over PAH emitted. Preliminary sampling results indicate that while quenching is a significant source of particulate emissions it is probably a relatively minor source of PAH emissions.

e) Emissions from battery stacks are due to leakage through oven walls into the battery heating flues and can be controlled by patching of cracks in the oven walls. Again, preliminary sampling results indicate that this is probably a minor source of PAH emissions.

f) Practically no information is available regarding emissions or control methodology used in by-product plants where recovery of material volatilized during coking takes place. Again, emissions are likely to be fugitive in nature and best control technology should involve prevention of leaks from pipes and use of tightly sealed tanks.

Coke oven emissions have been measured in terms of total particulate matter emitted, as well as its organic solvent soluble fraction and finally in terms of PAH, particularly B(a)P. The available emission estimates were developed from a rather limited data base and are therefore questionable. The intermediate uncontrolled emission factor given in Table IX represents a "best estimate", from all potential coke-oven sources combined, of 1.5 mg B(a)P per kg of coal charged, with the further assumption that 1.45 Mg of coal is required to produce 1 Mg of coke. In actual practice, routine measurements of PAH in fugitive coke-oven emissions is a very difficult technical problem and at present it would be practically impossible to contemplate regulation on this basis. However, reduction of particulate emissions, as measured visually or gravimetrically, is thought to result in corresponding reduction of PAH levels, as indicated by air quality data in the Canadian coke-oven cities of Hamilton and Sault-Ste.-Marie.[141-143]

2.3.4. Other Industrial Sources:

Other industrial sources thought to be of potential importance in Ontario from the point of view of PAH emissions are asphalt production and carbon electrode manufacturing. However, according to EPA estimates, B(a)P emissions from U.S. asphalt production for both paving and roofing applications do not amount to more than 10 kg per year and available statistical data regarding Ontario use of asphalt for paving purposes indicate that total yearly B(a)P emission from this source amounts to much less than 1 kg. This low figure seems to be confirmed by preliminary source testing data.[124]

The same preliminary source testing survey[124] also indicated relatively low PAH emission rates in Ontario carbon electrode manufacturing industry, although emission of the more volatile and polynuclear aromatics, particularly naphthalene was found to be appreciable (up to 200 g/hr).

These preliminary test results need further confirmation.

2.3.5. Heat and Power Generation:

Incomplete combustion of fossil fuels for the purposes of heat and power generation is one of the most significant potential sources of PAH pollution. Of the three basic types of fossil fuels, combustion of coal was found to result in the highest levels of PAH emitted. The burning of coal in residential furnaces was estimated to be responsible for the largest quantities of PAH emitted in the United States, among all the various source-types considered. Fortunately, however, coal-fired residential furnaces are nowadays almost nonexistent in Ontario, consequently they do not need to be considered in this report. On the other hand, about 90% of the fossil fuels used in Ontario for the generation of electric power is coal (about 8.8 million metric tons/year), thus representing a large potential source of PAH emissions. However, efficient combustion techniques, optimal operating conditions, good maintenance of the equipment and up-to-date pollution control systems usually combine to maintain PAH emissions from coal-fired power plants at a relatively low level, as indicated in Table IX. It is to be pointed out however that these Ontario estimates are based on emission factors developed by the U.S. EPA from relatively old data and its local verification

therefore may be advisable. Control of fine particulate emissions from coal-fired power plants is particularly advisable as it is known that PAH are preferably adsorbed on fine particulate matter. Venturi scrubbers, wet electrostatic precipitators and fabric filters can all be used for this purpose and are generally able to achieve a high degree of efficiency for removal of particulate matter, including, "fines".

Coal, oil or gas-fired intermediate size boilers are used in industry or commercial or public institutions such as schools and hospitals, to produce steam for space heating or other uses in industrial processes. Combustion efficiency, and therefore PAH production, again depends on the type of equipment used, the makeup of air-fuel mixtures, and the general maintenance and age of the equipment. The larger, more efficient industrial units are expected to be lower emitters of PAH then the smaller ones used in commercial and institutional establishments (e.g., Scotch-marine type boilers operating with low-pressure air-atomization of oil and premix burners for gas). Cyclones and wet electrostatic precipitators can be used for control of particulate (and presumably PAH) emissions, but cyclones are known to be less efficient in removing fine particles. Use of higher temperatures and excess air can also assure lower PAH production.

Residential furnaces may be an important source of PAH emissions due to the general lack of special control equipment. As mentioned previously, coal-fired residential furnaces, the major possible offenders, are practically non-existent in Ontario, thereby reducing the local seriousness of the problem. However,

even the oil- and gas-fired units may represent a potential hazard to health. Efficient furnace design, good maintenance practices and the use of clean fuels and repairing all leaks are the only means of reducing PAH formation and emissions from residential furnaces.

2.3.6. Municipal and Commercial Incinerators:

Disposal of solid wastes from households, industry, hospitals, restaurants etc. create an ever increasing problem, solved in part by reducing their volume through incineration. Large municipal incinerators usually burn waste material efficiently, at high temperatures, with large excess of air being supplied. In order to comply with air quality regulations, these incinerators are also equipped with water-spray towers or scrubbers and/or electrostatic precipitators which results in reduction of particulate and consequently PAH emissions.

On the other hand, the smaller commercial incinerators used in institutions and high-rise apartment and commercial buildings are usually characterized by inefficient combustion and are thus significant potential PAH emission sources. These units generally operate at lower temperatures with less excess air supply and usually afterburners constitute the only polluion control equipment used. The amount of PAH generated also depends of course on the type of wastes burned. As no provincewide data were available on the quantity of solid wastes disposed of by incineration, no emission estimates are given in Table IX. However, to indicate the expected order of magnitude of B(a)P emitted by these sources, one may refer to the emission factors mentioned in the 1973 IARC document[18], namely 0.02 - 3.3 mg B(a)P per kg of parti-

culate matter emitted from municipal incinerators and 58-180 mg. B(a)P per kg particulate matter emitted from commercial incinerators. Considering the 4.26 x 10^3 Mg of particulate matter emitted by Toronto area municipal incinerators in 1977,[144] a maximum of 14 kg B(a)P is expected to be emitted annually from these particular sources. Particulate matter emitted during 1977 from other, unclassified, incineration facilities located in Toronto, Hamilton, Sarnia and the Ottawa and Niagara regions amounted to 1.24 x 10^3 Mg according to ARB's Air Quality Model emission summaries.[144] Assuming that the highest abovementioned emission factor applies, a maximum of about 223 kg of B(a)P emissions may occur from these sources but actual emissions are probably considerably less.

On the other hand, one should not lose sight of the fact that incinerator residues also contain substantial amounts of PAH.[145] These residues are usually disposed of in landfills and may present an additional source of environmental contamination if leaching to ground - and surface waters occurs. This potential problem merits further investigation .

2.3.7. Automotive Emissions of PAH:

Combustion of gasoline and diesel fuel in internal combustion engines used in automobiles, trucks, buses, motorcycles etc., is another major potential source of PAH emissions. Emission characteristics of passenger cars and diesel engines are well documented,[12] while information concerning PAH emissions from aircraft, motorboat, snowmobile and lawnmower engines are almost nonexistent.[123]

a) Gasoline-powered automobiles: PAH formation in automobiles is dependent, on one hand, upon equipment design, operating

conditions, and wear, and, on the other hand, on fuel composition. Incomplete combustion and therefore increased formation and emission of PAH as well as carbon monoxide and other unburned hydrocarbons results from the use of fuel-rich carburetion, that is, less than stoichiometric air to fuel ratios. Thus, 30 times higher B(a)P formation was reported[146] with an air:fuel ratio of 10:1 than at a ratio of 14:1. Modern engine modifications have generally resulted in the use of leaner air:fuel mixtures and consequently lower PAH production. Higher PAH concentrations are produced in "cold starts" than during hot engine operation. Other factors resulting in increased PAH formation are related to wear of the engine and pyrolysis of heavy motor oil in the worn upper cylinder.[122,146] The nature and composition of combustion chamber deposits were also shown to influence the quantity of PAH emitted.[147] The effects of fuel composition on PAH emissions are well documented. As can be expected from what we know about their mechanism of formation, increased content of aromatics in gasoline inevitably leads to increased PAH formation.[148,149] PAH emissions are directly proportional not only to the original PAH-contents of the gasoline and motor oil used, but also to the number of carbon atoms found in aromatics.

The use of efficient emission control devices has helped to achieve a significant reduction of PAH levels emitted from modern automobiles. Catalytic converters are particularly effective in this regard, being able to reduce PAH emissions, by up to 99 percent.[123] Other control devices prevent or reduce evaporative fuel losses from gasoline tanks and carburetors, as well as control exhaust and crankcase emissions.

The emission factor of 16 µg/L has taken into account the effect of these various control devices as well as specific Canadian considerations. Thus, the emission factor of 9 µg/L given in Ref. 123 may be too low for Ontario climate, since cold temperatures (some 30°F difference between mean monthly temperatures in Canada and the U.S.) result in more frequent and longer cold starts and (because of snow and use of salt) in greater corrosion of control equipment. Also, emission controls in Ontario were introduced a year later (1969) and Canadian standards since 1975 have been considerably less stringent than U.S. ones. It was estimated that such differences could cause an increase in emission factor by about 80% hence the reported figure was adjusted to 16 µg/L.

(b) Motorcyles: the emission factor of 1.6 mg/L has been estimated by taking into account the proportion of vehicles powered by 2-cycle (38%, estimated emission factor: 2.9 mg/L) and 4-cycle (62%, estimated emission factor: 200 µg/L) engines, with a modest correction (about 30% increase) for cold starts. Combustion in 2-cycle engines, which use premixed lubricating oil and gasoline as fuel, is significantly less efficient than in 4-cycle engines, due mainly to mixing of exhaust gases with the fresh charge. Inefficient combustion and presence of heavy hydrocarbons (oil) in the fuel both contribute to the formation and emission of relatively large amounts of PAH.

Before 1978 there were no regulations requiring use of emission control equipment on motorcycles. For 1978-79 models, U.S. regulations require reduction of hydrocarbon and carbon monoxide emissions by some 30% and for 1980 by about

50%. As a result of this, larger 2-cycle engines have been abandoned and control systems similar to automotive equipment (even catalysts) are being considered. Both measures will result in reduction of PAH emission levels. A decision on similar Canadian legislation has not been made yet.

(c) Diesel-powered vehicles (such as trucks, buses and some passenger cars), although already required to meet stiff emission standards for gaseous pollutants (HC, CO, NO_x) and for smoke, are feared to emit higher concentrations of PAH than gasoline-powered vehicles.[150,151] In principle, the design of the diesel engine should result in more complete combustion of fuel, which is injected through atomizer nozzles into air compressed to high pressure and elevated temperature in the compression chamber. The high air-to-fuel ratios (assuring 20% to 600% excess of air at all times), the dispersion of the fuel into very fine droplets, and the high combustion temperatures should be conducive to relatively low rate of PAH formation. However emission of significant amounts of PAH may result from overfuelling, overloading, improper engine adjustment, and generally poor maintenance in the absence of additional exhaust controls. Again, 2-cycle diesel engines are known to emit more PAH than 4-cycle ones. Emissions of particulates depend also on the particular type of injection design and on the use of turbochargers.

In the U.S., new standards for particulate emissions from diesel automobiles are proposed, starting with 1981 models (0.6 g/mile). By 1983 all diesel engines should be covered with automobile standard dropping to 0.2 g/mile. If adopted in Canada, such standards, would significantly reduce PAH emissions from this source.

3. ENVIRONMENTAL PERSISTENCE AND FATE OF PAH:

3.1 Atmospheric Environment:

As mentioned in Chapter I, it is most likely that formation of PAH during combustion processes occurs in the vapor phase. However, condensation into fine particulate form and/or preferential adsorption onto fine particulate matter occurs shortly thereafter and, upon emission into the atmosphere, PAH compounds are almost invariably associated with aerosols of various types and sizes. Their ultimate fate and persistence in the atmospheric environment are determined on one hand by atmospheric physics and meteorological conditions affecting their dispersion, distribution between different atmospheric layers, atmospheric transport and fallout through wet or dry deposition, and on the other hand by the chemical changes they are most likely to undergo under atmospheric conditions, namely photooxidation, reactions with atmospheric oxidants and with other reactive species present in the atmosphere.

3.1.1. Physical Factors:

The importance of size and shape of particles associated with PAH compounds, as one of the factors determining the degree of health hazard they represent, has already been mentioned in Chapter I. Particle size and shape is also one of the factors determining their persistence and fate in the atmosphere. Apart from determining the residence time, this factor also plays a role in limiting the extent of chemical changes PAH undergo in the atmosphere. The atmospheric residence time of particles

less than 1 μm in diameter is estimated to be between 4 and 40 days in the absence of rainfall, while particles 1-10 μm in diameter usually remain airborne for 0.4-4 days under the same conditions.[152] Aggregation of smaller particles, multilayering of PAH on the surface and adsorption to the interstitial spaces all have the effect of shielding PAH from exposure to atmospheric reactants and light and thereby slowing and/or limiting the extent of their degradation.

Aggregation of small particles occurs through collisions brought about either by their Brownian motion, by air turbulence or by electrostatic forces. Smaller particles may also collide with larger ones due to differential gravitational settling and be swept up or "scavenged" by the large particles during their fallout. Thus, the particle size distribution of PAH-containing aerosols undergoes continuous changes during the aging process.

Atmospheric dispersion and transport of PAH occurs through passive diffusion and convection in moving air masses, as in the case of other pollutants adsorbed on airborne particulate matter, and depends largely on meteorological conditions. Assuming that dispersion of PAH closely parallels that of airborne particulate matter, the overall result is dilution, resulting in observed ambient PAH levels being significantly reduced relative to source strengths. On the other hand, atmospheric transport may result in the appearance of PAH in remote areas from their source, thus increasing the likelihood of exposure in those areas. Long-range transport of 20 different PAH has recently been documented,[153] confirming that they may survive relatively long atmospheric residence times without significant degradation.

Removal of PAH from the atmosphere occurs through the usual processes of dry and wet deposition. Dry deposition is brought about by gravitational settling or by impaction on obstacles such as rocks, buildings or vegetation. Particles smaller than 0.2 μm in radius may diffuse around obstacles, due to their thermal motion, thereby avoiding deposition by impaction. Wet deposition occurs by "rainout" or "washout" from within or under cloud-formations respectively. Although removal of PAH from the atmosphere results in the immediate reduction of their health hazard due to inhalation exposure, the removal process may, at the same time, result in contamination of soil, surface water and/or edible vegetation, thus perpetuating the potential hazard in different forms.

3.1.2. Chemical Degradation:

Chemical degradation of airborne PAH may be brought about by several mechanisms, the most significant ones being photooxidation, reaction with oxidant species such as ozone and hydroxy radical, and reaction with oxides of sulfur and nitrogen.

Photooxidation is probably the most important process contributing to the degradation of PAH in the atmosphere. As mentioned in Chapter I, this may involve generation and participation of the highly reactive singlet oxygen, as well as PAH radical cations in the process. While no kinetic data are available for the reactions of PAH compounds in the gas phase, photooxidation of PAH in solid and adsorbed

state has been studied by several authors under simulated atmospheric conditions. Thus, THOMAS et al.[23] reported a 60% destruction of B(a)P adsorbed on soot and exposed for about 40 minutes to light of an intensity equal to one-fourth that of the average July noon-time sun. At the same time, they observed formation of large quantities of acidic decomposition products, presumably resulting from the decomposition of other PAH compounds accompanying B(a)P. More recently, LANE and KATZ[60] reported half lives of 5.3 hours for B(a)P, 8.7 hours for B(b)F and 14.1 hours for B(k)F when exposed, as thin films, to simulated sunlight.

Reactions of PAH with ozone, in the solid state, were also investigated by LANE and KATZ[60] B(a)P was shown to be much more sensitive than B(b)F and B(k)F towards oxidation by ozone, as indicated by a half life of 0.62 hour, when exposed to 0.19 ppm ozone, vs 52.7 hours for B(b)F and 34.9 hours for B(k)F. It should be noted that these reaction rates refer to the surface layer only. In the presence of multilayered PAH the observed reaction rates will be lower due to the protective effect of the oxidized surface layer. Higher ozone concentrations were of course shown to result in faster reaction rates and the combined effects of light and ozone levels also result in enhanced degradation of PAH. Thus, were it not for multilayering and the shielding effect of particle agglomeration, present evidence indicates that the chemical half-lives of most PAH in the atmosphere would be limited to a few hours or days at most. Oxidation products such as quinones of B(a)P, BA, A, and DB(b,def)Chr have been detected in air extracts and other,

acidic degradation products are also likely to occur.

Another major oxidizing species found in urban air is the hydroxy radical (HO). Although rate constants for reaction of PAH with HO. radical have not been determined, the average half-lives of PAH undergoing this type of reaction has been estimated at about 10 hours by RADDING et al.[16], based on a reported HO. concentration of 10^{-14}M and the assumption that the reactivity of PAH is similar to that of ethylene ($K_2=10^{9.3}$ $L\,mol^{-1}\,sec^{-1}$).

Other atmospheric reactants that may play a role in the chemical modification and degradation of PAH include nitrogen oxides and sulfur dioxide. Reactions with these pollutant species may be of particular importance at higher temperatures and concentrations encountered in combustion plumes. The resulting nitro- and sulfonic acid derivatives of PAH have recently been detected[57,56] and further investigation of reaction rates, chemical properties and biological effects of reaction products seems to be warranted.

3.2 Aquatic Environment:

PAH enter the aquatic environment through precipitation and fallout from atmospheric aerosols, through domestic and industrial waste water discharges, through runoffs from city streets and other asphalt covered road surfaces, and through occasional oil spills. Typical concentrations may range from 10 ng/L in clear ground water to 100,000 ng/L in raw sewage.[154]

Due to their extremely low solubility in water, it is believed that the bulk of PAH will be present in adsorbed state, associated with suspended organic or inorganic particulate matter. Some PAH may be solubilized by synthetic detergents or natural surface-active agents such as humic and fulvic acids. Finally, a small fraction may also exist in true solution.

Natural removal of PAH from water bodies may occur by sedimentation, photooxidation or biodegradation. Removal in water treatment plants may be accomplished through mechanical means such as sedimentation, sand filtration and filtration on activated carbon, or through chemical means such as ozonation or treatment with chlorine compounds, particularly chlorine dioxide.

Sedimentation is the most important physical means both for natural cleaning of lakes and polluted rivers, and for purification of sewage. Usually about two-thirds of the suspended particles containing PAH may be removed by the combination of sedimentation and sand filtration, but occasionally a removal efficiency of up to 90% may be observed, especially in cases where PAH are associated with large particles.[154] Coagulation of suspended particulate matter by addition of chemical agents such as $FeCl_3$ usually helps in improving removal efficiency. Filtration through activated carbon has been shown in laboratory tests to result in elimination of up to 99% of PAH including those present in solution. However, in actual practice in water treatment plants, the result is close to 90% efficiency, due to loss of activity of the filter material in prolonged use.[154]

Photooxidation is the principal means of chemical degradation of PAH in natural water systems. Photodecomposition of B(a)P and BA was studied in detail under simulated environmental conditions, i.e., in solution in aqueous acetone, in aqueous suspension, and adsorbed on suspended inorganic particulate matter such as calcium carbonate or kaolinite.[64,155] These studies showed that photodecomposition of both compounds follows first order kinetics, the reaction rate depending mainly on the intensity of illumination, on the water temperature and on the concentration of dissolved oxygen. Factors such as pH and ionic strength of the surrounding medium had no apparent effect on the degradation process. In the case of particulate PAH it was shown that photodecomposition only occurs on the surface of the particles. In the case of particulate B(a)P the nature of the decomposition products is such that they form a protective shield preventing further photodecomposition, while the process is only slowed down in the case of particulate BA and complete decomposition is not prevented. Thus, the rate-limiting step in these cases seems to be mass transfer of the decomposition products away from the surface of the particles. Available data indicate that the primary degradation products of both PAH are quinones, which may further degrade under UV irradiation to unidentified, probably acidic, secondary degradation products. Reported half-lives under laboratory conditions ranged from 1.6 hours for BA in solution, to 15 days for B(a)P adsorbed on $CaCO_3$ at $5^{\circ}C$. While no corresponding data exist for other PAH, their photodecomposition is expected to be qualitatively similar and will depend on the abovementioned factors. Thus, it is

expected that, under environmental conditions existing in natural aqueous systems, the chemical half-lives of various PAH compounds will range from a few hours in shallow, clear, turbulent surface waters to as long as several years in deep, cold, turbid waters and bottom sediments, under anaerobic conditions.

Ozonation was shown to be the most effective chemical means of eliminating PAH from urban drinking water supplies.[156] However, due to more rapid ozone consumption by other organics and its rapid evaporation, water must be pre-purified for effective removal of trace quantities of PAH. Calculated half-lives of several PAH range from less than 10 minutes for DMBA to about 1 hour for B(a)P.[16] With an efficient pre-purification system and subsequent ozonation it is possible to obtain drinking water practically free of PAH (< 0.1 ng/L).[154]

Oxidative chlorination, as an alternative chemical means, is less effective in achieving complete removal of PAH. B(a)P is one of the most sensitive PAH compound in this regard and chlorine dioxide was shown to be particularly effective as an oxidizing agent. More than 90% of B(a)P present could be decomposed with this reagent within 2 to 3 hours. However the reaction rate decreased as the B(a)P concentration decreased and at a B(a)P concentration of 10 ng/L no further oxidation could be achieved. Other PAH compounds were shown to be less sensitive than B(a)P towards chlorination.[154]

Finally, elimination of PAH from aqueous systems may also occur through bioaccumulation in algae, aquatic invertebrates and fish, and there is some evidence that such aquatic organisms are able to metabolize and biodegrade PAH compounds. This subject will be further discussed in Chapter 4.

3.3 Soil and Vegetation:

Contamination by PAH of the upper layers of the earth including vegetation and forests occurs through fallout of airborne, PAH-containing particulate matter. The widespread nature of PAH-forming processes is shown by the fact that uncontaminated soil is indeed an exceptional occurrence. Typical B(a)P levels range from 1-10 µg/kg in uncultivated soil, remote from known sources, to about 100 µg/kg in fertilized soil and up to several mg/kg in heavily contaminated areas.[154] PAH may directly contaminate vegetation as well, and the waxy coating of some broad-leaved edible vegetables such as spinach, lettuce, leeks, etc. were shown to be particularly effective absorbents of these compounds.[157] Uptake by plants may also occur through the roots, and the possibility of biosynthesis of PAH by plant and soil microorganisms can not be excluded. All these facts are indicative of possible human health hazard by ingestion of PAH.

The degradation of PAH by photooxidation will continue to some extent when they have settled back on earth for as long as they are exposed to solar radiation. Thus, degradation half-lives of 1-2 days were observed for 3-methylcholanthrene, DB(a,h)A, B(a)P, DB(a,i)P and anthracene on the surface of oranges; however, the portions of these compounds that had penetrated into

the rind of the fruit were long-lived, with persistence half-lives of up to 200 days.[158] PAH may also be effectively degraded by certain soil bacteria[159] and, while actual data are unavailable. their persistence in the soil is expected to be ultimately determined by the abundance of such microorganisms. In the absence of sunlight and PAH-degrading bacteria and under anaerobic conditions PAH are expected to persist in soil for long periods of time. This conclusion has been substantiated by the fact that B(a)P and other PAH were found in soil samples obtained from a depth of 170 m and aged about 100,000 years.[154]

4. BIOLOGICAL PROPERTIES AND HEALTH EFFECTS

4.1 General:

While prolonged exposure to soot and coal tar has been known for over 200 years to result in occupational cancer in humans[1], it was not until the early 1930's that isolation and identification of B(a)P as a principal active ingredient in coal tar took place.[160] This focussed attention to the role PAH, as a general class, might play in the process of carcinogenesis, and the following decades saw a great deal of research undertaken in this area. Several hundred PAH compounds were synthesized and tested for carcinogenic activity in animal experiments, involving mainly application to the skin of mice. A number of these compounds were found to be active but an even larger number were shown to be inactive in these experiments.[161] Universally applicable structure-activity and dose-response relationships were found very difficult to establish, although several more or less successful attempts were made in this direction. Similarly, extrapolation of animal data to humans still remains a very controversial subject, and as of today, no single PAH compound has been conclusively proven to cause cancer in humans under normal environmental conditions.

While the exact nature of chemical carcinogenesis is far from being understood, the intensive research undertaken during the past few decades has resulted in the general acceptance of certain fundamental principles and the development of a common working hypothesis regarding the nature of the carcinogenic process. According to current, commonly held views, development of

cancer is the end result of a series of discrete events, well separated in time, each being subject to control and modification by a number of endogenous and exogenous factors. At least the following steps are involved in this complex process: 1) exposure to and entry of the potential carcinogen (pro-carcinogen) into the organism; 2) transport to target tissue 3) entry to target cell; 4) transport to the site of metabolism; 5) metabolic activation to the ultimate carcinogen; 6) reaction (formation of covalent bond) with the critical receptor; 7) formation of latent tumor cells; 8) development of tumor through the process of promotion; 9) transformation of a well-differentiated tumor into undifferentiated, malignant cancer. This complex process is summarized schematically in Figure 3. As mentioned, a great number of factors may come into play to control and modify any of the various steps of this process with the resulting cancellation of the cancer forming event. This explains the extreme difficulty of deriving valid theoretical predictions of carcinogenic activity based on limited knowledge of chemical structure and dosage of the potential carcinogen.

4.2 Definition of Terms:

Chemicals involved in the carcinogenic process may be classified as procarcinogens, proximate carcinogens, ultimate carcinogens, co-carcinogens, anticarcinogens, initiators and promoting agents.

Procarcinogens are often chemically and biologically inert molecules which have to be converted by a series of biochemical reactions into active matabolites in order to exhibit their carcinogenic potential. The majority of chemical carcinogens, including PAH, belong to this class.

Proximate carcinogens are biochemically modified derivatives of procarcinogens, intermediates in the chain of metabolic reactions between pro and ultimate carcinogens. They may exhibit some carcinogenic potency. B(a)P-7, 8-diol is considered to be an example of this class of chemicals.

Ultimate carcinogens are chemically and biologically reactive molecular species which can interact specifically with certain cellular components to yield critically altered macromolecules typical of the neoplastic or precancerous state.

Cocarcinogens are chemicals, not necessarily carcinogenic themselves, which, when administered simultaneously with another carcinogenic agent, results in significantly higher tumor incidences than either agent produces, when administered alone.

Anticarcinogens are chemicals which act in a sense opposite to cocarcinogens, reducing or eliminating tumor incidences.

Initiators and promoting agents: The use of these terms originally related to the so-called two-stage model of chemical carcinogenesis experiments on mouse skin. In these experiments, the initiator is applied on mouse skin in a single dose, resulting in the induction of benign or malignant tumors. However, the dose applied is such that no tumors ever develop at the site of applications without subsequent and repeated application of a promoting agent. Chemicals do not necessarily have to exhibit carcinogenic properties by themselves to act as initiating agents in two-stage mouse skin carcinogenesis experiments. Chrysene and benz(a)anthracene are examples of PAH initiating agents which are not carcinogenic per se on mouse skin. The best known promoting agent is a phorbol ester component of croton oil.

While the two-stage model of chemical carcinogenesis has not been applied experimentally to other systems than mouse skin, the relevant terminology is sometimes being used to describe more general phenomena related to chemical carcinogenesis.

4.3 Absorption, Tissue Distribution and Excretion:

Entry of PAH into the organism may occur through inhalation of polluted air or cigarette smoke, skin contact with soot, tar, and various industrial oils, and ingestion of contaminated food and water. Their absorption by lung, skin and digestive tract tissues constitutes the first step in the carcinogenic process. PAH are usually adsorbed on suspended particulate matter the particle size and surface characteristics of which largely determine the bioavailability and elution rate of PAH. While

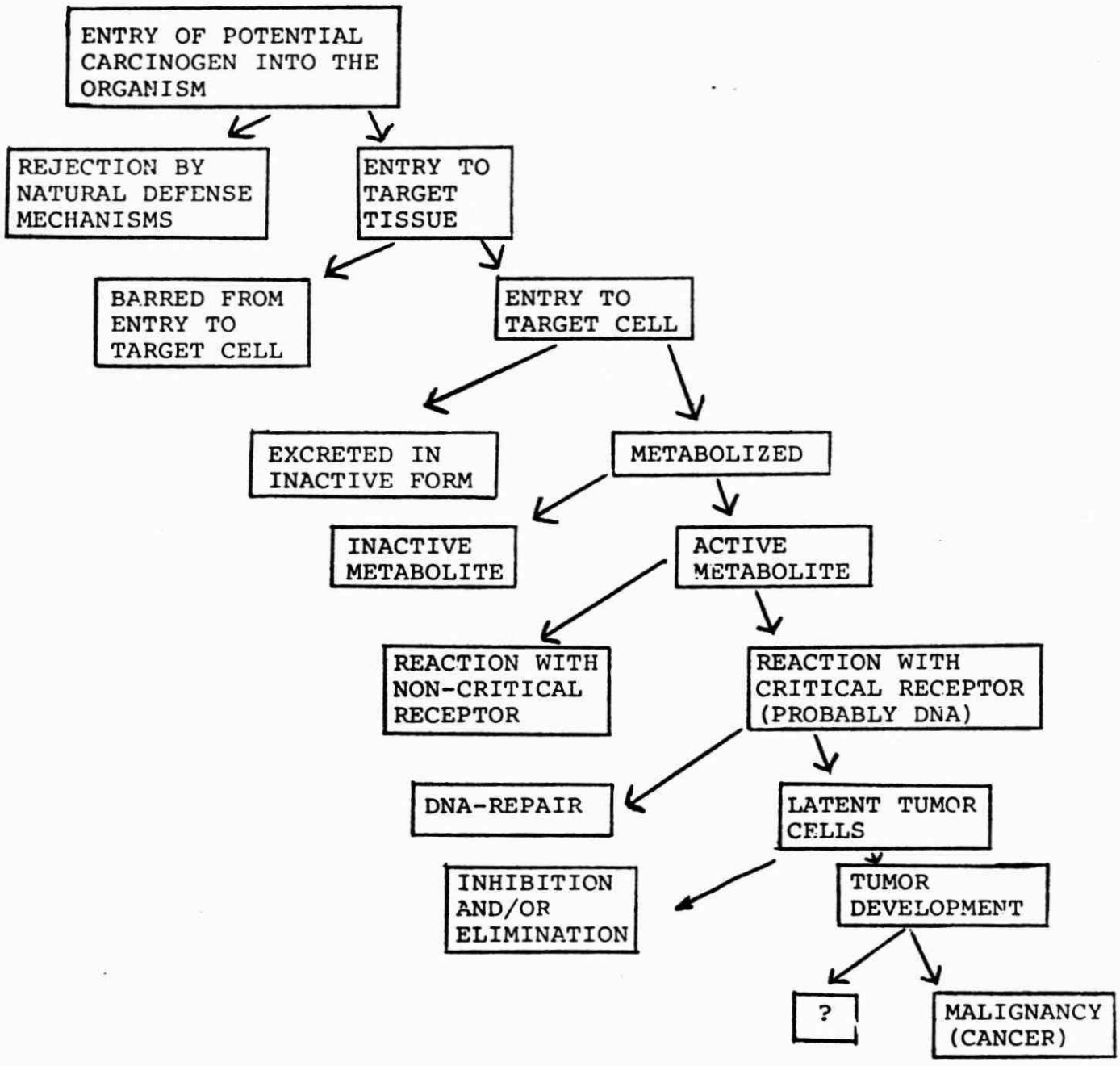


FIGURE 3: Schematic Summary of Chemical Carcinogenesis Process Steps

larger particles are usually entrapped within the upper respiratory passages when inhaled, particles of respirable size, that is 5 μm or smaller in diameter, are deposited deep in the lower respiratory tract.[162] The smaller the carrier particle and the larger its specific surface area, the slower the elution of PAH and the more prolonged the contact with the target tissue.[33] However, once eluted, absorption of PAH through the respiratory epithelium into the bloodstream occurs rapidly.[163] Absorption of PAH through the skin is a relatively slow process, allowing prolonged contact, and indications are that the potency of PAH as skin carcinogens is directly related to the extent of their absorption through the skin.[164] Intestinal absorption of PAH occurs relatively rapidly, by a mechanism involving physical adsorption to the intestinal wall and passive diffusion at exponential rate, once saturation level is reached in the gut.[165] Similar mechanism of absorption is thought to operate at other sites, such as adipose tissue.

Studies on tissue distribution of PAH indicate that initial accumulation occurs in the blood and liver, followed by a rapid decrease and subsequent accumulation in the bile and intestinal tract prior to elimination mainly in the feces.[163] Intratracheal administration results in local accumulation in the lung,[163] while on oral and intragastric administration significant and prolonged retention is also observed in body fat, mammary fat and glandular tissues.[166] The possibility of PAH accumulation in milk[167] and fetal tissues[168] raises special concern regarding the carcinogenic risk to offspring resulting from maternal exposure.

As for the excretion of PAH, it occurs mainly via the

bile and the feces, with lesser amounts being excreted in the urine.[162,164] While this general pattern seems to be independent of the animal species or routes of administration, the latter variable has a significant effect on the rate of elimination, oral, intragastric and intravenous administration resulting in rapid elimination, whereas intraperitoneal, intratracheal and dermal application results in much slower elimination. In addition to the above, elimination of PAH from the respiratory tract may occur through ciliary action and the inhibition of this mechanism by various irritants may result in a significant increase of risk or exposure to airborne carcinogenic PAH.

4.4 Metabolism and Metabolic Activation:

Metabolism of PAH by microsomal enzymes plays a critical dual role in determining their biological activity. On one hand, the main function of PAH metabolism is detoxification by conversion to more water-soluble derivatives, thereby facilitating their elimination, on the other hand metabolism of PAH may result in covalent binding of an active metabolite (the ultimate carcinogen) to a critical cellular macromolecule, which is thought to be the fundamental event in the induction of cancer. It is ironical that both activation and detoxification of PAH is effected by the same enzyme system known under various names such as mixed function oxydase (MFO) or aryl hydrocarbon hydroxylase (AHH). This enzyme system is found in all animal species examined so far, including insects, cold-blooded vertebrates, marine organisms as well as mammals.[169] It is concentrated mainly in the liver, but is also detectable in most other tissues including the lungs. It is located in the microscmal

fraction of the cells, needs oxygen and reduced nicotinamide-adenine dinucleotide phosphate (NADPH) for functioning, and is in fact a complex of several individual enzymes among which cytochromes P-450 and P-448 are the most prominent ones. The general function of this enzyme system is to convert lipophilic compounds into hydrophilic ones[170] for the purpose of eliminating these substances from the body in urine. This is accomplished through the formation of epoxides as metabolic intermediates, which can then isomerize to phenols, be converted to dihydrodiols by the action of the epoxide hydrase enzyme, or form mercapturates by conjugating with glutathione. Phenols and dihydrodiols can be further converted by conjugation, to form glucuronides and sulfates, which, along with mercapturates, are usually the ultimate, non-toxic metabolites excreted.[170] It is interesting to note here that carcinogenic PAH are excreted mainly as glucuronides, while mercapturates are the main excretion products of non-carcinogenic PAH.[171] During this metabolic process however, some of the active metabolites, probably the most active epoxides, may, under certain circumstances, react with cellular macromolecules and thereby cause induction of cancer. In fact there is a delicate balance between metabolic activation and detoxification which determines the biological activity of PAH.

Another common characteristic property of the AHH enzyme system is its inducibility: pretreatment of experimental animals with various chemicals, drugs, pesticides, such as phenobarbital, PCB and PAH themselves results in an increase of enzyme levels and their activities in various tissues. On the other hand, inhibition of this enzyme system is also possible by many chemicals.[12]

While there exists a general qualitative similarity of metabolic pathways of PAH in many species, there are also great quantitative differences at several levels, which makes extrapolations and predictions extremely difficult if not impossible. Going from the more general to the more specific levels, species related differences are the most obvious ones. Thus hamsters often show the highest and rats the lowest PAH-metabolizing activities.[172] In the case of B(a)P for example, the capacity of liver microsomes to activate this chemical to a mutagen is considerably greater in mice than in rats.[173] Not only does the activity of this enzyme system vary from species to species, but there are also significant differences between individuals of the same species, and this includes age and sex-related differences as well. On an even more specific level, enzyme activity usually varies from tissue to tissue within the same individual. Finally, indications are that the AHH enzyme system is chemical-specific, different constituents such as cytochrome P-448 and P-450 metabolizing various PAH at different rates. Over-and-above the inherent enzyme levels and activities, their inducibility by various environmental chemicals may also exhibit important species-, individual-, and tissue-related differences.[169] All these various factors have to be taken into account when considering metabolism of PAH in relation with their carcinogenic activity. The complexity of the metabolic process also points to the importance of studying it in as much detail as possible for individual PAH compounds.

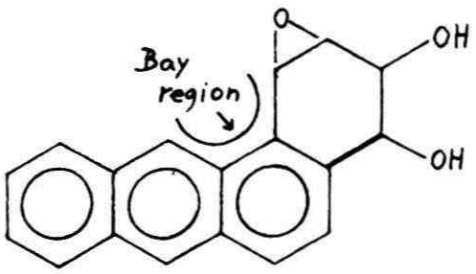
B(a)P has been the most widely studied PAH compound in this regard. Early metabolic studies were conducted in-vivo ,

while studies involving liver homogenates and various microsomal preparations were introduced subsequently, allowing more specific and detailed study of metabolic processes. Both in-vivo and in-vitro studies revealed a similar spectrum of a relatively large number of metabolites, arbitrarily classified into organic solvent soluble and water soluble ones. The former group includes several phenolic derivatives such as 1-, 3-, 6- hydroxy - B(a)P, three vicinal dihydrodiols namely the 4,5-, 7,8-, and 9,10- dihydrodiols and three quinones, namely the 1,6-, 3,6-, and 6,12- quinones, formed by the enzymatic or auto-oxidation of the corresponding phenols.[174,175] Water soluble metabolites of B(a)P comprise mainly glucuronide and sulfate conjugates of the various hydroxylated derivatives.[176] Epoxides have been proposed and were later detected as the common intermediates in the formation of both phenols and vicinal dihydrodiols[177, 178] and the importance of epoxide hydrase in catalyzing the formation of the latter metabolites has been emphasized.[175] Thus, the existence of B(a)P-4,5-, 7,8- and 9,10- epoxides as precursors of the corresponding dihydrodiols has been demonstrated.[179] Moreover, in the search for the ultimate carcinogenic metabolite of B(a)P, the importance of secondary metabolism of the vicinal dihydrodiols to diol-epoxide, was recognized.[98] At present there is strong evidence indicating that among the several possible stereoisomers of B(a)P-diol-epoxides it is the (+) - anti-configuration of the epoxide: 7α, 8β - dihydroxy - 9β , 10β - epoxy- 7, 8, 9, 10 - tetrahydro-B(a)P which is the ultimate carcinogenic metabolite.[180]

Other PAH compounds studied in some detail for their metabolic fate include BA, 7,12-DMBA, DB(a,h)A and MCA. With

unsubstituted PAH such as BA and DB(a,h)A early studies have shown that metabolism occurs primarily at their K-regions (positions 5 and 6 in the case of BA)[181]. However, non-K region phenols, dihydrodiols and their various conjugates have been detected as well.[182,183] In addition, some acidic metabolites resulting from ring cleavage were also detected.[184,185]

In contrast to the unsubstituted compounds, PAH bearing alkyl substituents, such as 7,12-DMBA[186], MCA[187] and 7- and 12-MBA[188] are primarily metabolized by hydroxylation of their alkyl side-chains. However, here again, both K-region and non-K-region phenols and dihydrodiols have been detected and recent studies[189] strongly indicate that vicinal dihydrodiols formed on the angular benzo-rings may be implicated in the metabolic activation of these PAH, resulting in the formation of the corresponding diol-epoxides as the ultimate carcinogens. As a generalization of studies involving testing the biological activities of different PAH metabolites in various test systems, the so-called, Bay-region theory[190] of carcinogenesis and mutagenesis evolved, according to which the ultimate active metabolites of PAH are those vicinal diol-epoxides in which the oxirane oxygen forms part of a "bay region"[191], e.g., in the case of BA:



As a matter of fact, one can show by quantum mechanical calculation[191] that opening of the oxirane ring of these bay-region diol epoxides results in the formation of the most stable carbonium

ions thus making these sites the most available ones for reaction with critical cellular macromolecules.

While, as we have seen earlier, in Chapter I, there are other possible candidate species as ultimate carcinogenic metabolites of PAH, it is the Bay-region theory which has so far received the most experimental support. As Bay-region diol epoxides clearly emerge as the most probable ultimate carcinogenic metabolites, it is to be emphasized once more that their formation and metabolic fate is under complex enzymatic control and that the role of epoxide hydrase may be particularly important in this regard. Thus, species such as mice, having high AHH activity but very low epoxide hydrase activity, may be much more susceptible than man to those toxic effects which are mediated by metabolically formed epoxides.[192]

4.5 Carcinogenicity of PAH:

4.5.1 In-vivo and In-vitro Testing Methods:

Ever since the realization of the role PAH might play in the development of human cancer, continuing efforts have been directed to developing sensitive and specific testing methods in order to pinpoint the identity and activity level of the causative agents. The earliest and most widely used testing method involves application of the chemical to the skin of experimental animals especially mice. Experimental variables include the dose applied, solvent used, as well as frequency and duration of application, while test results are expressed as percentage of animals with tumors, number and type of tumors and time elapsed to appearance of the first tumors. In the so-called

two-stage model of chemical carcinogenesis[193] a small dose of an initiating agent is painted onto the skin of mice in a single application, followed by repeated applications of a promoting agent. Other routes of administration include the oral route in feed or by gastric tube, as well as subcutaneous and intramuscular administration. Particularly important from the point of view of air pollution are the various experimental models involving exposure of lung tissues to potential PAH carcinogens. These include inhalation experiments, intratracheal administration as well as intravenous injection. The animal species most frequently employed in these experiments include the mouse, rat and hamster. Various primates and simians, guinea pig and fowl are also being used less frequently. Of most relevance to humans are of course the experiments with primates but the cost of testing is usually prohibitive. Hamsters are particularly suited to test the effects of PAH on lung tissue, while newborn mice are favored for general screening studies due to their extreme sensitivity to chemical carcinogens.

While in-vitro models of carcinogenesis were only developed relatively recently, they have provided much additional information and offer perhaps the most hope for the future, particularly in bridging the gap between animal and human studies. These in-vitro models involve observation of malignant transformation in various cell and organ-cultures on exposure to chemical carcinogens. Cell lines developed for these studies include hamster and mouse embryo cells, mouse prostate fibroblasts, epithelial cells from rat liver, mouse fetal skin and rat salivary gland, and a human osteosarcoma cell line. All

these cells, when transformed by chemical carcinogens, have the common property of developing into tumors when implanted into animals. Studies of the effects of PAH on organ cultures are relatively few and tissues used include rat and mouse prostate, and respiratory epithelia of hamster, rat, dog and monkey. Very recently, promising progress in developing suitable human tissue cultures for screening studies has been alluded to.[194]

4.5.2 Test Results:

As mentioned above, the most widely used in-vivo system for testing carcinogenicity of PAH is application to the skin of mice. Several crude PAH-containing environmental and commercial mixtures such as coal tar, creosote oil, shale oil, cutting oils as well as extracts of soot and airborne particulate matter were tested and found active in this system. Among the numerous individual PAH compounds tested by skin application to mice, B(a)P was consistently found to produce more tumors in a shorter period of time than did other PAH, with the possible exception of DB(a,h)A.[18,197] Other PAH found active in this test include DMBA, MCA, DB(a,h)P, DB(ai,)P, DB(a,e)P, B(b)F, B(j)F, with weaker or borderline activity having been found for BA, IP, B(e)P, and Chrysene.[18,197] Other species, such as rat, hamster and guinea pig were found to be less susceptible than mice, although DMBA was found to produce skin tumors readily in rats and high doses of B(a)P and DMBA were found to induce malignant melanomas on the skin of hamsters and guinea pigs respectively.[18]

The ability to act as initiators in two-stage carcinogenesis on skin of mice was demonstrated for BA, B(b)F, B(a)P, B(e)P, Chr., DB(a,h)A, DMBA and MCA.[18,197] Recent studies on the carcinogenicity of several B(a)P metabolites revealed that B(a)P -7,8 - dihydrodiol and its immediate metabolic precursor,

B(a)P - 7,8-epoxide, are complete carcinogens on mouse skin[195] while only initiating activity was demonstrated for one of the corresponding diastereomeric diol-epoxides.[196] The lack of activity of B(a)P-diol-epoxides as complete carcinogens on mouse skin is probably due to their high reactivity and short half-life.

Subcutaneous Injection of PAH to mice and rats usually results in the development of local sarcomas. Contrary to skin application, B(a)P was found to be less active than MCA or DB(a,h)A in this system.[18,197] Other PAH found active by subcutaneous injection include BA (although it did not induce tumors in rats at the dose tested), B(b)F, Chr., DB(a,h)P, DB(a,i)P, DMBA and IP.[18,197] Significant sex-related susceptibility was found when DB(a,h)P and IP were tested in one particular strain of mice.[198] Hamsters and guinea pigs are again less susceptible to tumor induction by PAH, relatively high doses of B(a)P and DB(a,i)P having been required to produce local tumors.[18] On the other hand, newborn mice are particularly susceptible to induction of distant tumors by subcutaneous injection of PAH,[199] development of hepatomas and lung adenomas having been observed on injection of BA, B(a)P, DB(a,h)A, MCA, DMBA as well as of extracts of airborne particulate matter.[200] Here again B(a)P appears to be less active than DB(a,h)A and MCA.[201] Recent studies on key B(a)P metabolites revealed that one of the diastereomeric bay-region diol epoxides of B(a)P, and B(a)P-7,8-diol, its immediate metabolic precursor, are respectively 40- and 15- fold more active than B(a)P in causing pulmonary adenomas in newborn mice,[202]

thus lending added support to the Bay-region theory of carcinogenesis.

Oral Administration of PAH either in the diet or by stomach tube, apart from the production of gastrointestinal tumors, also resulted in mammary tumors in rats, in the case of DMBA and B(a)P, and in induction of hepatomas and lung adenomas in mice, in the case of BA, B(a)P, DB(a,h)A and MCA.[18, 197]

Intravenous Administration of B(a)P and DMBA to rats also resulted in development of mammary tumors, while mice developed lung adenomas on intravenous administration of DB(a,h)A.[18, 197]

Intratracheal Administration of pure B(a)P has usually resulted in induction of respiratory tract tumors only when adsorbed on particles such as carbon, hematite and asbestos. The carcinogen - Fe_2O_3 combination was particularly successful in inducing a large number of cancers of the tracheobronchial tree in Syrian golden hamsters, which closely resembled naturally occurring lung tumors in humans.[203] Other PAH, apart from B(a)P which were found active by intratracheal instillation include DMBA, DB(a,h)A, MCA and DB(a,i)P. Other species used successfully in similar tests include rats and monkeys, apart from hamsters.[18, 197]

Inhalation Experiments , while conducted extensively with cigarette smoke[204] and occasionally with other crude environmental mixtures such as road dust,[205] gasoline smog[206] and chimney soot,[207] have practically not been used in the case of pure PAH, due to their time-consuming nature and to experimental difficulties in establishing exact and reproducible dosage of carcinogens. The only reported study concerned B(a)P - SO_2 mixtures,[208] inhalation of which over a period of more than a year resulted in production of squamous cell

carcinomas in 10% of the exposed rats but no tumors were observed in hamsters. While inhalation experiments are probably less useful than other models in screening the carcinogenic potential of PAH air pollutants, it would seem that more effort is justified towards developing their potential for use in establishing air quality criteria.

In-vitro Methods of testing for carcinogenicity offer the advantages of being less time consuming, requiring less material and allowing more direct means of observing and evaluating enzymatic and other biochemical influences at the cellular level. In studies with hamster embryo cells in culture, malignant transformation was induced by treatment with B(a)P, DMBA, MCA, Db(a,h)A and BA.[209] K-region epoxides and cis-dihydrodiols of BA and DB(a,h)A were shown to be more active than the parent compounds in this test,[210] in contrast to results obtained in-vivo, which may be explained by the relative instability of the epoxides when applied to skin. Recent studies indicate that an overwhelming majority of the known carcinogens are able to induce malignant transformation in this system whereas none of the non-carcinogens tested were able to do so.[211] Thus, this system may provide an efficient pre-screen for chemical carcinogens. The effect of various inducers of epoxide-forming enzymes and of inhibitors of epoxide-hydrase enzyme resulting in enhancement of the degree of malignant transformation could be demonstrated with mouse embryo fibroblasts.[212] The ability to induce malignant transformation of mouse prostate cells was well correlated with in-vivo carcinogenic activity in the case of several PAH including B(a)P, MCA, DMBA and DB(a,h)A.[213] More recent studies on derivatives of 7-MBA using a mouse prostate derived cell line indicated that non-K-region dihydrodiols were significantly more

active than the parent compound in this test system, while K-region dihydrodiol, which cannot be directly metabolized to a vicinal diol epoxide, was devoid of any activity.[214]

The use of organ cultures would seem to be particularly attractive, as offering practically the only direct means of comparing human and animal target tissue responses on exposure to carcinogenic PAH, which could not be safely given to living human subjects. Unfortunately however, reports of such studies are completely lacking, presumably due to serious experimental difficulties. Moderate success has been achieved nevertheless in cultures of mouse prostate[215] and rodent respiratory epithelia,[216] where B(a)P, DMBA and MCA induced characteristic morpholgic transformations.

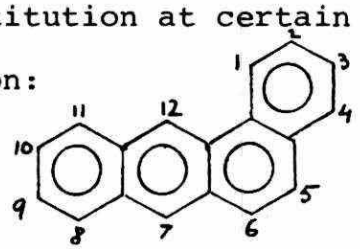
4.5.3 Structure-Activity Relationships:

Structure-activity relationships, as a means of predicting the carcinogenic potency of PAH were sought since the earliest investigations. On a purely empirical basis it was soon realized that an angular phenanthrene-like moiety is the most basic structural requirement for manifestation of carcinogenic activity in PAH. Furthermore, it became apparent that a certain optimal molecular size and complexity was also required for enhanced carcinogenic properties.[11] Thus, while phenanthrene itself is inactive, addition of further benzenoid rings to the parent nucleus results in a number of highly active compounds. On the other hand, compounds resulting from the linear extension of anthracene appear to be devoid of activity.[11] Compounds resulting from the fusion of four benzenoid rings in angular arrangement do not appear to be of sufficient molecular size and complexity to exhibit pronounced carcinogenecity. Of the six possible arrangements only three,

namely chrysene, benz(a)anthracene (BA) and benzo(c)-phenanthrene exhibit weak or borderline activity under most conditions of testing. Penta-and hexacyclic PAH on the other hand include a number of highly potent compounds such as B(a)P, DB(a,h)A, DB(a,e)P, DB(a,h)P, DB(a,i)P, DB(a,l)P, B(b)F and B(j)F. Only a few heptacyclic and even fewer of the more complex PAH are known to possess carcinogenic activity.[11]

Several numerical scales of carcinogenicity have been devised, the most widely used one being the so-called Iball Index[217] expressed as a ratio of the percentage of animals with tumors and the time required to appearance of the first tumors. However, the large number of possible experimental variables, such as animal species, strain, age, sex, diet, mitotic cycle, site and vehicle of administration, duration and frequency of exposure, etc., make it imperative that interpretation of results should proceed with extreme caution and that indices should only be compared when standard procedures have been applied to large numbers of standardized animals.[218]

Such valid comparisons are possible in the case of the isomeric methyl-substituted benz(a)anthracenes. In this case it was shown that methyl-substitution at certain favorable positions of the parent hydrocarbon:



(namely at positions 7 > 6 > 8 ≅ 12 > 9 in decreasing order of potency) can lead to considerable enhancement of carcinogenic activity.[218] Even more potent compounds are obtained on dimethyl substitution at the 6,8 -, 8,9-, 7,8-, 8,12-, and 7,12- positions the latter one being one of the most potent PAH skin carcinogens

known.[218] On the other hand, methyl-substitution in the angular benzo ring (1,2,3,4 positions) results in partial of total l ss of carcinogenic activity,[219] a tendency which is not altogether surprising in view of the more recently developed Bay-region theory of chemical carcinogenesis. Similar relationships apply in the case of B(a)P.[197,220] It was also shown that substitution at the 7-position of BA by highly polar groups such as -0H, -C00H, $-0CH_2COOH$ or $-SO_3H$ completely abolishes carcinogenic activity.[11] However, when the polar -OH and -COOH substituents are not directly linked to the aromatic system, but through interposition of one or two $-CH_2$-groups, carcinogenic activity is then retained.[11] This seems to indicate that the deactivating effect of these polar substituents is due to electron-withdrawal from the aromatic system.

Attempts to correlate carcinogenic potency with the electronic structure and properties of PAH originated as early as 1938[221] and have been later developed in full detail by theoretical chemists in France[51] and elsewhere.[49] Among the several theories proposed, mention should be made of MASON[222], based on the hydrocarbon-protein binding hypothesis of chemical carcinogenesis. According to this theory, energy matching should occur between the highest occupied and the first or second unoccupied molecular orbitals of proteins and PAH for carcinogenic activity, the required energy level differences between the two levels being restricted to an approximate range of 3.0-3.3 electron volts. Experimental confirmation of this theory, however was tenuous at most.[223] The theory developed by the PULLMANS[51] and the DAUDELS[10] was more successful in correlating carcinogenic

activity with electronic properties of PAH. This theory paid particular attention to the so-called K-, L-, and M- regions of individual PAH compounds, distinguished by their chemical reactivity described in terms of electron densities, bond orders, free valences and localization energies, as defined in Chapter I of this report. High reactivities in the K-regions were thought to be particularly important for reactions to occur with critical receptors for carcinogenic activity, while reactions at the L- and the metabolically active M-regions usually led to deactivation of the molecules. In fact it was shown that for carcinogenic activity, the K-region must have a particularly high bond order and an ortho-localization energy smaller than 3.31β (β being the Huckel resonance integral), while at the same time para-localization energy at the L-region must be greater than 5.66β [224, 51]. While reasonably good correlations between electronic indices and carcinogenic activity of various PAH was obtained on this basis, several exceptions and discrepancies are known to exist [218] and the main shortcoming of this theory was that metabolic activation was not taken into account. Furthermore it was shown that reactions of K-region derivatives with cellular macromolecules did not lead to products which were identical to products of <u>in-vivo</u> reactions between PAH carcinogens and the same macromolecules [96-98].

Current investigations, as mentioned previously, lend much experimental support to the Bay-region theory of chemical carcinogenesis, according to which Bay-region diol epoxides act as the ultimate carcinogens. The most significant experimental evidence in support of this theory is provided by the demonstration that reaction products of synthetic Bay-region diol-epoxides with DNA are chemically indistinguishable from those produced by <u>in-vivo</u> reaction of the parent PAH with the same cellular macro-

molecule.[225] Theoretical support of the validity of this theory is provided by quantum chemical calculations showing that among carbonium ions derived from all possible PAH diol epoxides those being part of a bay-region are the most stable ones.[191] Thus, it seems that the optimum conditions of high reactivity and relative stability are best met for these PAH derivatives, making them the most likely ones to react with critical cellular macromolecules.

4.5.4 Dose-Response Relationships:

Study of dose-response relationships has especially important implications with regard to environmental risk assessment of carcinogenic PAH. PAH compounds are distinguished among chemical carcinogens by the fact that even single applications of minute amounts of the most potent members of the series are sufficient to induce cancer in experimental animals. Thus, single applications of microgram quantities of B(a)P[226] and DB(a,h)A[227] were shown to induce skin cancer in mice, whereas amounts of the order of 1 mg of several other PAH such as DB(a,h)P, DB(a,i)P and B(b)F[18] were sufficient to induce tumors by single administration either topically or subcutaneously.

Obviously, detailed knowledge of the effects produced at low dose levels comparable to environmental concentrations would be very desirable for establishing environmental criteria of safety for PAH. Yet, while a large number of studies were conducted in order to derive meaningful dose-response relationships, the nature of the dose-response curves obtained is such, that establishment of safe dosages based on them is next to impossible.[12]

While approximate linearity of response has been demonstrated at high dosages of several PAH, the curve usually flattens and becomes concave at the low dose range. This deviation from linearity of the dose-response curve at low dosages is subject to several possible interpretations. One of these is that at very low dosages, it is practically impossible to obtain statistically reliable data without enormous numbers of animals. For example, in order to demonstrate a 0.01% tumor incidence, a non-negligible risk in human terms, an experiment using 50,000 animals at a single dose level would be required for statistical reliability: a proposition which is clearly absurd. Other interpretations of the concave curvature of the dose-response curves associate it with the existence of threshold dosages.[228] This again is impossible to verify experimentally, since, on one hand, the absence of tumors induced with low-doses of a carcinogen might be interpreted as a chance occurrence and, on the other hand, the existence of a threshold could not be denied on the basis of tumor incidence, because it could be a spontaneous rather than induced occurrence at low doses. Thus, trying to prove the existence or non-existence of threshold doses seems to be pointless. Nevertheless, on a purely speculative basis, interpretation of the flat portion of dose-response curves as being due to a variation of individual thresholds and sensitivities within the same species of experimental animals seems to be a plausible one.[228] According to yet another possible interpretation,[229] the two portions of the dose-response curves correspond to the induction and promotion phases of chemical carcinogenesis, with no threshold for tumor induction, but a demonstrable threshold dose existing for the promotion phase. Again, from the practical point of view of establishing safe doses,

this sort of distinction seems to be pointless. The slope of the linear portion of the dose-response curve is usually taken as indicative of the sensitivity of the species. Another very important variable from the point of view of environmental risk assessment and possible establishment of "safe" doses is the duration of the experiment and the time required to appearance of the first tumors. Usually, the lower the administered dose, the longer is the time required and such observations at several dosage regimens, including low ones, might be extrapolated in a manner to obtain "safe" dosages at which the time of appearance of the first tumors would significantly exceed the expected life-span of individuals in the species in question.[230] This sort of extrapolation is again subject to the same statistical uncertainties as outlined above. However, given adequate epidemiological and environmental exposure data, this kind of approach may still be the best one for human risk assessment and establishment of acceptable environmental criteria. In the absence of such data, one has to rely on the best available animal data for human risk assessment, even though it must be realized that the resulting extrapolation is, by its very nature, a very crude and approximative one due to statistical uncertainty, variation of sensitivity between species and presence of a variety of possible co-factors in the human environment.

4.5.5 Co-and Anticarcinogenic Effects:

Co-carcinogenesis is defined as an increase in total tumor yield on simultaneous administration of two agents over the sum of tumor yields when each agent is administered alone.

Conversely co-administration of an anticarcinogenic agent with a carcinogen results in the partial or total inhibition of tumor production by the latter. Several PAH compounds, including Pyrene, B(e)P, B(g,h,i)Per, and Fluoranthene, which are not carcinogenic by themselves, were shown to significantly enhance the carcinogenic activity of B(a)P when administered in combination with the latter.[193,231] Other co-carcinogens include several alkylnaphthalenes present in cigarette smoke.[232] The co-carcinogenic effect of tobacco smoke, asbestos and ionizing radiation has been amply documented in human studies.[18]

While both tumor promoters in two-stage carcinogenesis experiments and co-carcinogens have the same end effect of enhancing tumor production, they probably have different mechanisms of action and should not be confused. As a matter of fact, several of the above-mentioned PAH co-carcinogens do not have tumor promoting activity.[193]

Although the mechanism of co-carcinogenesis is not known, factors such as diet, stress, hormonal balance, exposure to environmental irritants and infection have all been suggested as being able to modify the carcinogenic process.[12]

As for inhibition of carcinogenesis, several PAH were shown to exhibit anticarcinogenic activity when administered in combination with B(a)P to C57 strain mice.[233] These included B(e)P, BA, B(g,h,i)Per, as well as anthracene, phenanthrene, pyrene, perylene, fluoranthene, chrysene and coronene.[233,234] Also, co-administration of the di-and hexahydro derivatives of DB(a,h)A and MCA along with the respective parent compounds resulted in total

or partial inhibition of the expected carcinogenic response.[233] Other anticarcinogenic agents that where shown to be effective inhibitors of PAH-induced tumor development include several commercial antioxidants[235] such as BHA, BHT, ethoxyquin, sulfur-containing anti-oxidants[236] such as disulfiram and dimethyldithio-carbamate, several natural and synthetic flavonoids,[237] Vitamin A,[238, 239] and some naturally occurring isothiocyanates[240] present in the diet. There is experimental evidence indicating that these various anticarcinogenic agents act by influencing the metabolism of PAH, by inhibition or induction of specific hydroxylating enzymes.[237, 241]

Thus, co-carcinogens may act by increasing the level of active metabolites of carcinogens whereas anticarcinogens probably have the opposite effect on the production of active metabolites. The possible co- and anticarcinogenic effects of other environmental constituents present along with PAH have always to be taken into account when considering the health risks due to the latter compounds.

The above mentioned experimental data also indicate the probability that some PAH may act both as co-carcinogens or anticarcinogens, depending on the animal species or strain used.

4.5.6 Correlation Between Carcinogenesis and Mutagenesis:

Positive correlation between chemical carcinogenesis and mutagenesis implies that a basic similarity exists in the molecular mechanism triggering the two processes. According to the somatic mutation theory, carcinogenesis, as well as mutagenesis, is the result of an alteration in the structure of the

genetic material of cells, namely DNA, caused by direct interaction with the chemical. While this concept has been generally accepted in the case of mutagenesis, its applicability to carcinogenesis has been disputed in the past, mainly because of failure of early attempts to demonstrate mutagenicity of many known carcinogens, including PAH. According to the alternative, non-mutational theory, carcinogenesis is the result of an indirect modification of gene expression by binding of the chemical carcinogens to proteins involved in genetic control mechanisms. However, as soon as the importance of metabolic activation became generally recognized, development of more sensitive mutagenicity testing methods ensued, showing definite correlation between the carcinogenicity and mutagenicity of most carcinogens including PAH and thus lending much experimental support to the somatic mutation theory of carcinogensis. As a result, mutation assays are now being proposed and used as preliminary screening tests for environmental carcinogens.

Among in-vitro mutagenesis test systems the Ames assay[242] is the most widely used one. The test uses several sensitive bacterial testing strains of Salmonella typhimurium the genetic material of which has been modified in such a way that they cannot usually survive and grow in the absence of the amino acid histidine. Such modifications can be brought about either by substitution of one nucleotide in the DNA chain for another, or by deletion or addition of a nucleotide to certain specific regions of the DNA. The different tester strains of S. typhimurium were obtained through these different means of modifications. Now, chemical mutagens have the property of being able

to revert or back-mutate these histidine-dependent strains to histidine-independent ones by the same mechanism that formed the original mutants. Thus, one can distinguish between so-called base-pair substitution mutagens and different frame-shift mutagens by using different tester strains, such as TA1535 and TA100 in the former case, and TA1537 and TA98 in the latter case. The sensitivity of strains TA98 and TA100 toward chemical mutagens has been greatly enhanced through the addition of so-called resistance transfer factors (R-factors) to the bacteria.[243] In order to study the effect of metabolic activation, the test may be carried out in the presence or absence of appropriate enzyme preparations, usually rat liver homogenate containing AHH. In this way, one can distinguish between mutagens that require metabolic activation and those that do not.

To date, over 300 chemicals, both known carcinogens and non-carcinogens were tested by this system and its efficiency is such that it gave positive results for 90% of the carcinogens tested, whereas negative results were obtained for at least 85% of the non-carcinogens.[244] Furthermore, good quantitative correlation was also shown between carcinogenic and mutagenic potency of various PAH tested in this system, including MCA, B(a)P, DB(a,i)P, DB(a,e)P, DB(a,h)A, BA and B(e)P.[245] This system has been used extensively in studying the mutagenic activity of various PAH metabolites,[246] most extensively those of B(a)P,[247,248] with the objective of identifying the ultimate carcinogenic metabolites. The results found provide additional evidence indicating that Bay-region diol epoxides are most likely to

be the ultimate carcinogenic metabolites. Moreover, a high degree of stereospecificity could be demonstrated by investigating the mutagenic potency of various stereoisomeric B(a)P derivatives in this system.[249] The Ames test has also been used to assess the mutagenicity and carcinogenic potential of crude environmental mixtures such as cigarette smoke condensates[250] and organic extracts of airborne particulate matter.[251] Results of the latter type studies indicate that much of the activity is due to compounds other than PAH, that do not require metabolic activation.

Other in-vitro mutagenicity testing systems employ mammalian somatic cells in culture. While these are technically more difficult and time-consuming to carry out, they offer some important advantages over the bacterial systems: in contrast to the Ames test which relies on reverse mutations, chemical mutagens induce forward mutations in these systems, which are probably more relevant to the process of carcinogenesis. Moreover these cell systems represent more typical targets for both mutagenic and carcinogenic processes thus allowing the establishment of more direct correlation between the two. The most widely used test system of this type employs the V79 Chinese hamster cells derived from male lung tissue, which are sensitive to certain cytotoxic agents affecting the surface membrane (ouabain) and nucleic acid synthesis (8-aza-guanine).[252] Temperature-sensitive cells derived from Chinese hamster ovary are also used.[253] Chemical mutagens have the property of inducing resistance in the cells against the noxious effects of elevated temperature and cytotoxic agents. Since these cells lack the capacity for metabolic

activation of PAH, the test was modified to provide this capacity by the addition of special "feeder" cells such as lethally irradiated rodent fibroblasts,[254] Syrian hamster kidney cells[255] or rat liver microsome preparations.[256] The mutagenicity of a number of PAH tested in this system correlated well with their respective carcinogenic activity.[253] The carcinogenic PAH were active at all three genetic loci mentioned above, with DMBA showing the highest activity, which persisted at a concentration as low as 0.01 µg/ml, while PAH generally considered as non-carcinogenic, such as pyrene, phenanthreene, chrysene and BA did not show any significant mutagenic activity.[253] Testing of a number of B(a)P-metabolites in this system again pointed to the bay-region diol epoxides as the most likely ultimate carcinogenic/mutagenic derivatives.[248,257] Furthermore, the recent demonstration, that B(a)P and its 7,8-diol can induce both malignant, cancer-like transformation and mutagenesis (ouabain-resistance) in the same normal diploid hamster embryo cells,[258] lends added support to the somatic mutation theory of carcinogenesis.

Still other <u>in-vitro</u> techniques that were proposed for the screening of potential mutagens and carcinogens include the analysis of chromosomal aberrations,[259] sister chromatid exchanges[260] and DNA repair synthesis[261-263] induced in various cell cultures. While some encouraging results were obtained, particularly by using the latter method, these techniques are not nearly as successful as the formerly mentioned ones in predicting the carcinogenecity of chemicals and need further development in this sense.

In-vivo mutagenicity tests include the induction of dominant lethal mutations[12] in male rodents which are measured by enumeration of early fetal deaths and preimplantation losses in mated females. However, it is felt that this test is not particularly sensitive.[264] Another in-vivo mutagenicity test uses the fruit-fly Drosophila melanogaster, in which a wide range of carcinogens were shown to be able to induce various types of chromosome damage.[265] In this test, a reasonable correlation between mutagenicity and carcinogenic activity was found in the case of DMBA, MCA and BA.[266] It is also interesting to note that the parent PAH exhibited a higher specificity toward genetic loci in their mutagenic activity than their respective, more active but less selective K-region epoxide derivatives.[266]

Two additional in-vivo mutagenicity tests need to be mentioned here, which, taken together, were almost as successful as the Ames test in distinguishing between carcinogens and non-carcinogens.[267] One is the so-called micronucleus[268] test which detects chromosome damage of the rapidly dividing erythroblastic cells of mouse bone marrow. Fragments of damaged chromosomes are often left in the cytoplasm of these cells where they can be readily recognized as micronuclei. The other, more recently developed method is the so-called sperm abnormality assay[269] which is based on observation of abnormal sperm head morphology following exposure of male mice to many mutagens, carcinogens and teratogens. It is proposed that these two assays, along with the Ames test, would provide a relatively inexpensive, rapid and powerful system to predict the carcinogenic potential of pure chemicals as well as complex environmental pollutants.[270]

4.5.7 Human Carcinogenesis and Exposure to PAH:

Evidence, implicating PAH as causative agents of various types of cancer in man both at occupational and at environmental levels, is provided with various degrees of certainty by a large number of epidemiological studies. However, it should be stressed that all these studies involved exposure not only to complex PAH mixtures, but usually also to trace metals, particulate matter and irritant gases such as SO_2 and NO_x, and therefore the modifying effects of other potential carcinogens, co-carcinogens, tumor initiators and promoters can never be entirely discounted when attempting to evaluate the human carcinogenic effects attributable to PAH.

In occupational settings, development of skin cancer in humans was shown to result from prolonged exposure to soot,[1,271] coal tar,[2,272] creosote oil,[272] various lubricating and cutting oils,[273] and heavy oil[274] and petroleum distillates,[275] all of which contain significant amounts of PAH and all of which are now recognized as human carcinogens. Although the association between exposure to these PAH-containing industrial materials and human skin cancer is clearly established in a qualitative manner, the available data are not sufficient to establish dose-response relationships, to estimate relative risks for various exposed groups or to identify any specific PAH compound as the causative agent.

More extensive epidemiological data are available on workers exposed to fumes from coal carbonization and gasification processes in coke oven plants and gas production plants.

In these cases very few measurements of actual PAH and more specifically B(a)P levels are available but establishment of a crude dose-response relationship, ie.., estimation of relative risk at various exposure levels is nevertheless possible. In the case of gas production workers two to three fold excess incidence of lung cancer over that occurring in the general rural, nonsmoking population was demonstrated.[276,277] The reported exposure levels corresponded to approximately 1 $\mu g/m^3$ of B(a)P[278] which is orders of magnitude greater than usual ambient atmospheric levels. A fourfold excess of bladder cancer was also observed in the group of heavily exposed gas-works employees as compared with workers in the low-exposure group.[277] Perhaps the most extensively studied group of workers where exposure to PAH-containing emissions may be significant involves coke plant employees.[279-282] The available data indicate that a definite correlation exists between increased cancer risk and both level and duration of exposure to coke oven emissions. Thus, coke oven workers in general experienced a 34% higher incidence of cancer of all sites, compared to the overall steelworker population.[280] The relative risk of lung cancer was found to be two-to threefold for coke-oven workers in general,[279,280] about fivefold for topside workers[279] and seven-to fifteenfold for those topside workers with five or more years of full-time exposure.[280-281] Even more definite dose-response relationships could be established, particularly for non-white coke-oven workers, when cumulative exposure of the cohort in terms of coal tar pitch volatiles was correlated with cancer death rates.[282] Apart from the excess incidence of lung cancer, a

five- to eightfold excess of kidney cancer could also be demonstrated among coke oven workers.[280,282]

Other occupations shown to be at increased risk of developing lung cancer due to potential exposure to PAH-containing emissions include roofers, heat treaters, taxi-drivers, cooks, machine shop workers, plumbers and steel industry workers.[283,284]

The effect of nonoccupational exposures to PAH is much more difficult to correlate with development of human cancer than that due to occupational exposures. A major part of this difficulty stems from the fact that the effect of cigarette smoking, which has been recognized as the main causative factor of human lung cancer,[285] is very difficult to separate from the effect of ambient exposures to relatively low levels of PAH. While cigarette smoke itself contains significant amounts of various PAH, it has been shown that their combined effect is not additive but is dependent on the presence of other co-factors such as arsenic, SO_2, fluorides etc.[135] The same situation also pertains to ambient PAH which are always accompanied by other pollutants, providing another complicating factor in evaluating their effects. Further problems arise from having to take occupational exposures into account, from the inhomogeneity of the populations, from geographic and temporal variations of the type and level of pollution within the same metropolitan area and from the fact that correlation between present pollution levels and mortality may not represent the effect of exposures that occurred many years earlier.

Despite these various difficulties, a number of epidemiological studies were undertaken in attempts to characterize the importance of various ambient environmental factors in the etiology of human cancers. Significant differences in cancer and particularly lung cancer mortality rates between urban and rural populations, amounting roughly to a factor of two, were convincingly demonstrated by early studies.[286-289] While strong correlation was found in all relevant studies between cigarette smoking and lung cancer death rates, such differences cannot be accounted for entirely by differences in smoking habits. Similar conclusion was reached by studies on migrant populations whose lung cancer death rates in their adoptive country remained correlated, to a certain degree, with earlier exposure to pollution in their former country of residence.[12] Consequently, it has been suggested that air pollution, acting synergistically with cigarette smoking is responsible for the observed excess of cancer incidence. However, various attempts to correlate cancer incidence rates with various indices of air pollution taken in their entirety as recently summarized in an extensive EPA study,[197] do not provide clear-cut evidence that this is indeed the case. While positive correlation between excess lung cancer incidence and solid fuel consumption,[290] suspended particulates, trace elements,[291] PAH and/or B(a)P[292-3] were found by some studies, others[294-7] found no such correlation. One widely quoted study,[298] based on regression analysis of U.S. lung cancer death rates in relation to tobacco sales and ambient B(a)P concentrations, concludes that an increase in average B(a)P level by 1 ng/m^3 corresponds to a 5% increase in lung cancer death rates.[12] However, this study was criticized[297] for being based on questionable B(a)P and tobacco sales data.

Another quantitative estimate[299] for the possible effect of ambient B(a)P levels is based on linear extrapolation of the effect observed at one high exposure level among British gas workers and concludes that each ng/m^3 of B(a)P may cause 0.4 extra lung cancer cases per 100,000 population per year. This estimate may be criticized for being based on a very crude method of extrapolation from a single high exposure level. Furthermore, the use of B(a)P levels, as an indicator of ambient exposure to carcinogenic PAH in general, is itself a questionable practice on several grounds: (a) the composition and B(a)P level of PAH emissions is highly variable depending on their sources, and consequently no constant and significant correlation can be expected between the levels of B(a)P and that of most other PAH present; b) B(a)P levels cannot account entirely for the observed carcinogenic and mutagenic activity of organic extracts of suspended particulate matter.

In conclusion, while occupational exposure to PAH-containing industrial mixtures and emissions has been conclusively proven to be a causative factor in the development of human cancer and its effect can be characterized in a quantitative manner, the degree of involvement of ambient PAH could not be unequivocally demonstrated by currently available data. Nevertheless, even if the degree of cancer risk due to ambient PAH levels is impossible to assess accurately at the present time, epidemiological and animal data provide sufficient indication that such risk does indeed exist at a non-negligibile level.

4.6 Other Toxic Effects:

Although other toxic effects of PAH, as reflected by the amount of relevant literature, are certainly overshadowed by studies of their carcinogenic potential, the fact that environmental and occupational exposures to PAH may also result in various other toxic manifestations should not be left unnoticed. One particularly serious consequence of the mutagenicity of PAH is the possibility of permanent genetic damage which may affect subsequent generations. However, this possibility has so far not been documented. In humans, both occupational and ambient atmospheric exposures to PAH and particularly B(a)P were linked with increased incidence of chronic bronchitis. Occupational exposures to PAH-containing materials such as coal tar pitch, creosote, asphalt, high-boiling petroleum residues and various lubricating, cutting, and coolant oils, which are known to produce skin cancer, have also been reported to cause various skin disorders such as contact dermatitis, cutaneous photosensitization, folliculitis, acneiform lesions and hyperpigmentation.

Corresponding animal studies more directly demonstrated the involvement of specific PAH compounds in similar toxic effects. Thus, intratracheal applications of B(a)P or DMBA to hamsters induced chronic inflamation of the lung and extensive damage to the bronchial epithelium before appearance of tumors. As for skin exposure, it is well known that application of many carcinogenic PAH to mouse skin, also leads to destruction of sebaceous glands. Cutaneous photosensitization, observed in man is paralleled by the phototoxicity of PAH against the ciliate: Paramecium caudatum

Other toxic manifestations of PAH in experimental animals, that are not overtly paralleled by human observations

but which may be indicative of the mechanism of their toxicity, are directed mainly against normally proliferating cells. Thus, in young mice the principal target organs on administrations of high doses of MCA were the thymus, the spleen and the lymphoid system.[305] In rats the toxic effects of DMBA were directed mainly against the bone marrow,[306] lymphoid tissues[307] and the testis,[308] particularly those germinal cells actively synthesizing DNA. Several investigators reported the immunosuppressive effects of PAH and a good correlation of this effect with their carcinogenic potency, indicating the involvement of the immune system in the process of carcinogenesis.[309] The ability of PAH to induce drug metabolizing enzymes, and particularly aryl hydrocarbon hydroxylase, (AHH) has been mentioned earlier. The importance of this effect in the production of toxic metabolites in various AHH-containing tissues such as liver, lung, skin, testis, bone marrow, intestine, etc., has to be emphasized. On the other hand, it can be shown that tissues lacking AHH activity are usually also resistant to the toxic effects of PAH.[197]

4.7 Bioaccumulation, Biodegradation and Ecological Effects:

A number of field and laboratory studies indicate that PAH can be taken up from the environment by a variety of lower and higher organisms. Thus, bioaccumulation of B(a)P was demonstrated in a large number of soil- and aquatic bacterial species.[159,310-313] Various marine and freshwater invertebrates,[314-318] fish,[318,319] algae[316,318] and higher plants[159,320] were also shown to accumulate PAH from the environment and significant differences in accumulated PAH

levels were observed between polluted and non-polluted environments. Recently observed PAH levels in fish-eating birds[321] such as herring gulls indicate the possiblity of their accumulation via the food-chain. However bioaccumulation of PAH is not expected to present nearly as serious an ecological problem as for example the organochlorine compounds, as indicated by the extremely low levels of PAH found in fish,[322] relative to the latter compounds, and due to the fact that PAH can be much more effectively metabolized and biodegraded by various strains of bacteria[311,312] and fish.[318,319] As a matter of fact, it has been shown that various soil bacteria are able to metabolize about 50 to 90 percent of B(a)P and other PAH present in 4 to 5 days.[159,312] Aquatic bacteria appear to be less efficient in this regard.[311,323,324] It has also been shown that trout liver microsomes were 15 to 30 times as active as rat liver microsomes in metabolizing B(a)P.[325] The fact that metabolism in lower organisms may result in the formation of toxic PAH metabolites is reflected by the toxic effects PAH exert on these organisms. Thus, high concentrations of several PAH were shown to inhibit the growth of various bacteria.[316,317] PAH also exhibitied adverse effects on sludge microorganisms, as measured by significantly reduced oxygen uptake.[323] On the other hand, some PAH such as B(a)P and DB(a,h)A were shown to stimulate bacterial growth,[326] a seemingly contradictory result.

Adverse effects on exposure to PAH have been reported in invertebrates, fish and amphibian species as well. Such effects included hyperplasia and incipient tumors in earthworms, planaria and snails, abnormal growth in sponges, photo toxicity

in Paramecium, indicating involvement of toxic photooxidation products, and inhibition of growth and maturation of housefly larvae.[16] Various skin tumors in bottom-feeding fish species of the Great Lakes have been reported recently,[328,329] which seems to be related to industrial pollution involving PAH. Hyperplastic reactions and tumors have also been produced experimentally in such amphibian species as the newt[330] and the clawed toad[331] on application of B(a)P and MCA.

The effects of PAH on plants have not been studied extensively. While phenanthraquinone was reported to be toxic to various varieties of bluegreen algae,[332] other PAH compounds such as fluoranthene, BA, IP, B(a)P, and DB(a,h)A were shown to enhance the growth of Chlorella vulgaris and other algal species.[333] Growth promotion of higher plants, such as rye, radish and tobacco, by PAH was also observed.[333] While direct phytotoxic effects of PAH on higher plants have not been observed, such effects were reported to occur on exposure of the PAH-treated plants to sunlight, indicating that photoxidation products of PAH may produce plant damage.[334] The demonstrated fact that higher plants, including those used for human consumption, may accumulate carcinogenic PAH by absorption through the roots[159] and selective adsorption on the leaves[157] is giving cause for concern and should be given more attention than it now receives.

5.0 SAMPLING AND ANALYTICAL METHODOLOGY

The need for environmental monitoring and control of PAH is quite obvious in view of the potential emissions and carcinogenic-mutagenic hazard some of these compounds represent. The great complexity of PAH-containing environmental samples presents a tremendous challenge to the monitoring effort. Efficient sample collection, extraction and separation techniques, as well as specific and sensitive methods for detection and quantitative determination are all essential steps in the monitoring process. Particular attention must be paid at each step to avoid possible sources of error due to the physical characteristics and chemical complexity of the environmental PAH samples. The available techniques and possible sources of error will be briefly described and discussed in the following sections, for each of the above-mentioned steps in the PAH-monitoring process.

5.1 Sample Collection -

As airborne PAH may be present either adsorbed on the surface of suspended particulate matter, in aerosol form, or in the vapor phase, any efficient sampling device must, in principle, be capable of collecting all three forms of PAH. This criterion is especially important in the case of source sampling, where a significant proportion of PAH is present in the vapor phase.[20,337] Other factors to be considered when planning sampling strategy and selecting sampling equipment are (1) the sample size, which will depend on the expected concentration as well as the sensitivity of the analytical method; (2) the selection of sampling sites to

ensure that samples collected are truly representative of actual source strengths or environmental concentrations; (3) the need for collecting size-fractionated samples to provide specific information on the proportion of PAH present in respirable form; (4) the need to avoid losses due to volatilization, photo-degradation and chemical degradation.

5.1.1 Source Sampling -

Several methods have been developed for sampling PAH at their sources. These are various modifications of the EPA Method-5 sampling train,[335] which itself is based on the earlier method developed and used by HANGEBRAUCK and collaborators.[122] The EPA Method-5 uses a heated, stainless steel probe to sample stack emissions under isokinetic conditions. The sample is passed through a heated pre-conditioned glass fiber filter to remove particulate matter, and the filtered gases are then passed through a series of four ice-cooled impingers, the first two containing 100 mL of water, the third one empty and the fourth one containing 200 g of silica gel. The drawback of this train is that the more volatile PAH compound may pass through to the silica gel where they may get irreversibly adsorbed or pass through unchanged. The recovery efficiency of the EPA Method-5 train was reported[336] to average about 85% but even lower efficiency figures were also mentioned.[337] Modifications to improve collection and/or recovery efficiency include the use of organic solvents, such as xylene, in the impingers,[336] the use of Tenax[337] or XAD-2[338] adsorbent cartridges and the use of cyclones for particulate collection and size fractionation. Typical flow-rates range from

10 to 30 liters per minute and convenient sample size requires 6-8 hours collection. In one method developed for collection of PAH from automobile exhaust[339] the filtering system includes a fiber-glass pad placed ahead of a glass fiber filter to effect a gross particle-size fractionation of the particulate matter, while the gaseous components and aerosol passing through the filter are captured in a Chromosorb-102 adsorbent trap.

5.1.2 Ambient Air Sampling -

Of all the methods utilized for the collection of B(a)P and other PAH from the atmosphere, collection with high-volume samplers using glass fiber filters has become the most widely used one.[340] This method relies on the high (over 99%) collection efficiency of the glass fiber filter for airborne particulate matter of 0.3 μm diameter or larger size and the premise that the overwhelming majority of PAH is present in the particulate phase. The sampling unit consists of a filter holder and gasket, filter adapter assembly and a heavy duty suction pump capable of continuous operation for extended periods of time. The unit is protected from debris and extreme weather conditions by a suitable housing which, at the same time allows unrestricted air flow. A calibrated flow-meter should also be provided. At typical flow rates of 1.4-1.6 m^3/min and with average particulate loading in ambient air, approximately 200-300 mg of particulate matter can be collected from 2000-2400 m^3 of air during a 24-hour period. Depending on the sensitivity of the analytical method used, this quantity may or may not be sufficient for the desired purpose and pooling of several samples may be necessary.

While the high-volume sampling method is very efficient in collecting airborne particulates, its main drawbacks are that very fine particles and PAH present in the vapor phase escape collection on the glass fiber filter and that evaporative losses, consistent with the non-negligible vapor pressures of PAH, may also occur after collection, especially in the presence of air-flow.[24] In order to reduce these collection losses, the use of back-up adsorbents such as polyurethane foam has been suggested.[341] A combination of silver membrane and glass fiber filters, along with Chromosorb-102 or XAD-2 adsorbent has recently been developed for personal sampling of workplace atmospheres.[342]

A variety of other samplers are available to collect particles of various size ranges in order to assess the mass distribution of PAH according to particle size of atmospheric aerosols.[343] The best known of these are the Andersen size-fractionating cascade impactors, incorporating five to seven stages to collect particles of various aerodynamic diameters. Various cyclones and dichotomous samplers are also available for the same purpose.

One common source of possible error during collection of ambient PAH is due to their interaction with airborne oxidants and other reactive species, resulting in their chemical transformation.[445] This is an area of study requiring further investigation.

5.1.3 Sampling of Aqueous Systems -

As in atmospheric sampling, the expected concentration of the particular pollutant is usually one of the main limiting factors in determining the choice of sampling method in the case

of aqueous systems as well. Thus, grab sampling is usually satisfactory in the case of effluents or raw sewage containing appreciable amounts of PAH. This may be followed by organic solvent extraction, or purging and head-space analysis for the more volatile components. However, in the case of aqueous systems containing PAH in trace quantities only, sampling of large volumes is required which necessitates the use of special techniques. An instrument employing on-site liquid-liquid extraction has been described,[344] but is of doubtful practical value for routine analysis of PAH due, on one hand, to the slow flow-rates (20-35 mL/min) necessary to establish an equilibrium distribution between the aqueous and organic phases and, on the other hand, to the fact that water solubility limits the range of suitable solvents. Another approach to sampling large volumes of aqueous systems for trace contaminants such as PAH is the use of adsorbent cartridges to trap the contaminants. While activated carbon is the best known adsorbent for PAH, it does not allow quantitative re-extraction and the result is poor overall recovery. The use of macroreticular resins, such as XAD-2,[345] and of porous polyurethane foams[346] have been proposed as alternative adsorbents, with approximate recovery rates of 90% being achieved in laboratory experiments.

5.2 Extraction of PAH from the Sampling Matrix -

Solvent extraction is the most common way of recovering PAH from the sampling matrix. Other alternatives are thermal desorption and vacuum sublimation. The most frequently used solvents are benzene,[341,347-350] cyclohexane[351-353] and methylene chloride.[354-356] Other

solvents being used for this purpose include methanol,[357] acetone,[358] chloroform[359] and carbon disulfide.[360] Extraction efficiency, speed, selectivity for PAH, and reproducibility are some of the important factors to consider when selecting a particular solvent. Thus, while benzene is a more efficient extractant than cyclohexane, it is also less selective towards PAH, consequently benzene extracts usually contain much more polar, non-PAH material than cyclohexane extracts. The toxicity of benzene is a further factor arguing against its use. Methanol is one of the best extractants, allowing near-quantitative extraction of most PAH compounds, but the large quantity of polar co-extractives usually necessitates additional clean-up before actual analysis can be attempted. Methylene chloride provides a good compromise between efficiency of extraction and selectivity towards PAH. As extraction efficiency may vary not only from solvent to solvent but also from compound to compound when using the same solvent, determination of this parameter for each compound becomes important if accurate quantitative analysis of particular compounds is desired. Otherwise, lack of knowledge of extraction efficiency may be another source of error. The usual extraction technique involves refluxing the collection medium with the chosen solvent in a Soxhlet extractor.[343] The procedure requires 4 to 12 hours of extraction, at the boiling point of the solvent, for maximum extraction efficiency, with the possibility of some decomposition taking place. The more recently developed ultrasonic method[353] of solvent extraction has the advantages of being more rapid (requiring 10-15 minutes), requiring low temperatures (0-25^{o}C) and being more precise (relative standard

deviation: ± 1.3% vs. ± 26% for Soxhlet extraction)[343]. The disadvantage of the ultrasonic extraction technique is that it may produce unfilterable suspensions of the residual particulates and the filter material itself.

As mentioned earlier in this section, thermal desorption and vacuum sublimation are the alternatives to solvent extraction of PAH from the sampling matrix. The thermal desorption technique is usually used in connection with gas chromatographic separation and flame ionization or electron capture detection. This technique involves heating of the collection medium, in a desorption device, to elevated temperatures (about 250-300°C), stripping the eluted PAH compounds with an inert carrier gas (usually nitrogen or helium), and either trapping them in a cold (dry ice or liquid N_2) column containing a packing identical to the one used subsequently for GLC separation[361], or directly injecting them onto a packed column through a special gas sampling valve.[362]

One advantage of the thermal desorption technique is that by eliminating the use of solvents it also eliminates evaporative losses during the solvent concentration step. Another advantage is that it increases the sensitivity of the method by using the entire sample for the analysis, not just a small aliquot. Finally, the possibility for automation is another advantage of the thermal desorption technique. Its disadvantages are: a) the possibility of decomposition or rearrangement of PAH compounds at high temperatures; b) the difficulty in establishing recovery efficiency; c) the possibility of poor resolution due to peak broadening; d) the fact that this technique does not allow replicate injections of the same sample.

The technique of vacuum sublimation has been developed and is routinely used by Japanese investigators.[363,364] It involves evacuation of ice-cooled sublimation flasks, containing the collection medium, to 0.001-0.005 mm Hg, subsequent heating of the flask to about 300°C for a period of approximately 40 minutes and dissolution of the sublimate, which is deposited in the cold part of the sublimation tube, in a small amount of benzene.[365] This technique offers the advantages of speed, elimination of evaporation losses by eliminating the solvent concentration step, and the possibility of automation. While recovery and reproducibility were reported to be good,[363] this finding has been disputed.[366] This technique also suffers from the disadvantage of allowing possible decomposition or rearrangement of PAH compounds at the high temperatures used in the process.

5.3 Sample Clean-Up and Analytical Separation -

The tremendous complexity of some typical PAH-containing environmental mixtures such as crude oil, cigarette smoke condensate or automobile exhaust condensate usually necessitates extensive clean-up before quantitative analysis can be attempted. The purpose of this step is to separate a PAH-enriched fraction from most other classes of accompanying impurities which would interfere with the final analysis. Once the PAH fraction is isolated, the individual compounds and isomers have to be separated from each other as completely as possible for accurate quantitative determination.

The extent of clean-up required depends of course upon the degree of complexity of the particular sample. While complete

clean-up and analytical separation may be achieved in a single step in some cases, others may require multiple fractionation and separation steps. The importance of the process can be best appreciated by taking into account the fact that over 900 PAH compounds could so far be separated and identified in cigarette smoke condensates.[367]

5.3.1 Clean-up Procedures -

The first step in the clean-up of complex environmental mixtures usually involves fractionation into acidic, basic and neutral fractions.[368] This is accomplished by treating the original organic extract sequentially with aqueous alkaline and acidic solutions which will allow the extraction of the acidic and basic components of the mixture respectively, leaving behind about 90% of all PAH in the neutral fraction, along with other neutrals such as paraffins, olefins, aromatics, etc.[360]

Another approach, used either alone or in combination with acid-base extraction or column chromatographic clean-up, involves partitioning between immiscible solvent pairs having different affinities towards PAH and some of the more or less polar components. For example, partitioning between aqueous methanol (usually 4 parts methanol and 1 part water) and cyclohexane[369,370] allows separation of PAH from the more polar impurities which preferentially pass into the more polar aqueous methanol phase. On the other hand, separation of PAH from less polar components such as paraffins can be accomplished by partitioning between non-polar solvents such as cyclohexane or heptane and polar ones such as nitromethane,[369,149] dimethyl formamide[371] or dimethyl sulfoxide,[371,372]

PAH preferentially distributing themselves in the more polar phase. Dimethyl sulfoxide was shown to be particularly useful for this purpose, allowing over 90% recovery for most PAH.[372] After dilution with water, PAH can be re-extracted from this solvent into cyclohexane to facilitate further work-up and analysis.

Column chromatography is another widely used procedure for the clean-up of PAH-containing samples prior to actual analysis. Again, it is usually used in combination with other clean-up procedures. Some of the most popular adsorbent materials for column chromatography of PAH include neutral alumina, silicic acid Florisil and various polymeric gels such as Sephadex or Bio-Beads. The sample to adsorbent ratio usually varies between 1:100 and 1:1000. The efficiency of separation is determined by such factors as column length, particle size and activity of the adsorbent and polarity of the eluent. Maximum separation can be achieved by slowly and gradually increasing the polarity of the eluting solvent system. For example, using silicic acid as adsorbent and various ratios of benzene : petroleum ether as eluent, PAH are eluted in the 1:3 benzene: petroleum ether fraction.[367] Other solvent systems used for elution include cyclohexane - benzene,[373] cyclohexane-ether,[374] ether-pentane,[375] benzene-methanol or benzene[359] alone. Gel permeation chromatography using various grades of Sephadex[368,354] or Bio-Beads,[367] which is based both on molecular size exclusion and adsorption processes, is particularly useful for the separation of PAH according to number of rings and number of alkyl substituents.

While column-chromatographic clean-up offers the advantages of better selectivity and separation efficiency compared to the acid-base extraction and solvent partitioning procedures, it also has several disadvantages, namely that it is time-consuming, requiring the use of automatic fraction collectors, and that decomposition, photooxidation or artifact formation may occur during the relatively long residence time on the column.

5.3.2 Analytical Separation Procedures -

5.3.2.1 Thin-Layer Chromatography (TLC) -

Although TLC has been proposed and used as an additional pre-fractionation procedure[352], its resolving power does not allow separation of a large number of compounds from complex environmental mixtures. However it can be quite successful in separating individual PAH compounds, including isomers, from less complex mixtures and from pre-fractionated samples. In contrast to column chromatography, it offers the additional advantages of being rapid, inexpensive and reasonably reproducible.

Silica gel[352], alumina[376], cellulose[377], cellulose acetate[378] or combination of some of these materials[379,380] have been used as adsorbents in this technique. Usually a 0.1 to 0.5 mm thick layer of adsorbent, spread evenly on a glass plate, is used as the chromatographic medium. The sample is spotted and the chromatogram developed with a solvent system usually consisting of various mixtures of benzene and cyclohexane, hexane and ether, or other suitable solvents. Resolution can be improved by varying the composition of the developing solvent system, by using mixtures

of sorbents and by using two-dimensional development. The best resolution was achieved with a composite TLC plate of aluminum oxide- 40 percent acetylated cellulose (2:1), using two-dimensional development with hexane: ether (10:1) in one direction and with n-propanol:acetone:water (2:1:1) in the second, perpendicular, direction.[380] Recoveries ranged from 84% to 92% for various individual PAH compounds, with good separation of B(e)P from B(a)P, and B(b)F from B(k)F.[380]

Spots corresponding to individual PAH may be scraped off the plate and eluted for quantitation[380] or may be quantitated in-situ by using a fluorescence scanning device.[378,381] Among the drawbacks of the TLC method is the fact that individual spots may sometimes actually correspond to a mixture of two or more closely related compounds. Furthermore, one has to pay particular attention to the possibility of photodecomposition and special dark-room techniques are therefore required.

5.3.2.2 Gas Chromatography -

Modern gas chromatographic techniques allow excellent resolution of most PAH, including isomeric ones, at convenient speeds. Complete separation of phenanthrene from anthracene, benz(a)anthracene from chrysene, B(e)P from B(a)P and B(b)F from B(k)F are some of the most difficult problems in gas chromatography as well as in other separation techniques. Suitable liquid stationary phases must meet exacting requirements of high separation efficiency, high-temperature stability for extended periods of time, and low column bleeding. A number of

such liquid stationary phases have been investigated and used for separation of PAH, including the OV series of silicone fluids (OV-1,[382] OV-7,[383] OV-17,[384] OV-25,[384] OV-101,[385] in ascending order of polarity), the SE series (SE-30,[386] Se-52,[387] SE-54),[388] Dexsil-300,[389] Dexsil-400,[390] polymetaphenoxylene (PMPE),[384] apiezon L[386] and apiezon W.[391] Some nematic liquid crystals have also been proposed as stationary phases,[392] offering excellent resolution, but these have serious problems of column bleeding at the recommended elevated temperature, resulting in a loss of resolution after a few runs.[343]

Columns used in gas chromatography can be classified as packed columns and glass capillary columns, with the latter ones further subdivided into support-coated and wall-coated open tubular capillary columns (SCOT and WCOT). SCOT capillary columns are pre-coated usually with barium carbonate as support before applying the liquid stationary phase. Solid supports used in packed columns include Gas Chrom Q, Universal support, Chromosorb W.[384] Packed columns vary in length from about 2 m to 10 m and their performance in terms of number of theoretical plates generated for B(a)P varies from about 1000 to 10,000,[384] while capillary columns may reach a length of 50 m, with number of theoretical plates ranging from 30,000 to 150,000 While packed columns offer the advantage of higher sample capacity with reasonably good resolution, capillary columns allow the achievement of the highest possible resolution, at the expense of reduced sample capacity.

The use of OV-7 liquid stationary phase in packed columns was found to be very satisfactory for the routine analysis of PAH in environmental samples,[383] while capillary columns using

SE-54 as the stationary phase was shown to allow base-line resolution of all the above-mentioned, difficult-to-separate PAH isomers.[388]

A rapid and convenient procedure was recently reported by KARASEK and collaborators[393] for the analysis of complex organic mixtures on airborne particulate matter. The column packing used in this procedure is an ultra-thin, thermally treated layer of Carbowax 20M deposited on exhaustively acid-washed 100/120 mesh Chromosorb W.[394] Although this packing did not resolve B(a)P from B(e)P and BA from Chrysene when used in a 10 foot x 2 mm i.d. glass column, it did eliminate the pre-separation step and proved useful in survey and trend-type studies involving a number of organic compounds along with PAH.

5.3.2.3 High-pressure Liquid Chromatography (HPLC):

HPLC is clearly superior to conventional liquid chromatography and offers an attractive alternative to gas chromatography as an efficient method of separation for the routine analysis of most major PAH usually found in environmental samples. While good resolution of common PAH mixtures may require as long as two to five days using conventional liquid chromatography on long alumina columns, the time required for separation, using HPLC, is usually reduced to 20-100 minutes, depending on the degree of resolution desired.[352]

Compared to gas chromatography, which undoubtedly continues to be the most effective technique for complete resolution of complex environmental mixtures particularly when capillary columns are used in combination with mass spectrometric detection,

HPLC nevertheless offers a number of advantages in terms of cost, sensitivity, reproducibility and ease of operation. The analysis time per sample is shorter for HPLC when compared to GC-MS. The much lower operating temperatures (below 80°C) of HPLC eliminate the possibility of artifact-formation on the column, cause no severe limitations on the nature of the column packing due to thermal stability requirements, and at the same time achieves better stability and reproducibility of column performance. HPLC also has at least an order of magnitude larger sample capacity, which, when coupled with the high sensitivity of fluorescence detection, lowers the effective detection limit significantly for most PAH. Thus, the detection limit of B(a)P has been reported to be about 1 ng per injection by GC,[395] whereas HPLC lowers its detection limit to 1 pg.[396]

The advantages of HPLC have been greatly enhanced by the recent development of various octadecylsilylated (ODS) columns of micro particle size. These columns, operating on the reversed-phase principle, are highly selective for separating isomeric PAH such as B(a)P and B(e)P, which are difficult to separate by normal GC techniques. Separation efficiency of HPLC can be further enhanced by the use of gradient elution techniques including automatic solvent programming devices. Among stationary phases used in HPLC mention should be made of Zorbax-ODS,[341] ODS-permaphase,[397] Vydac,[398] Bondapak-C_{18},[399] Corasil-C_{18},[400] and cellulose acetate.[401] Separation of PAH on these columns is accomplished by reverse-phase mechanism,[341,397] using various mixtures of water and methanol or acetonitrile and water,[398] preferably in the gradient elution mode. The more traditional HPLC techniques use Alumina,[404] Porasil T[402] or Spherosil[403] as stationary phases in the normal mode of separation

by adsorption, with non-polar hydrocarbons being used as the mobile phase. When compared with three other reverse phase HPLC column packing materials, Vydac 201TP was found to achieve the best resolution.[405] On elution with a 3:1 mixture of acetonitrile and water, isocratically, at a pressure of 105 Kg/cm^2 and a flow rate of 1 mL/min, B(a)P was well separated from B(k)F as well as B(e)P, and Chrysene was partially separated from BA, the complete analysis requiring less than 25 minutes.[405]

5.4 Detection and Quantitation:

5.4.1 Ultraviolet-visible Absorption Spectrophotometry:

This has traditionally been the most widely used method for the detection and quantitative analysis of PAH. As mentioned in Chapter I, most PAH have strong and characteristic absorption bands in the U.V.-visible region of the spectrum, with molar extinction coefficients typically in the 10^4-10^5 range at 150-300 nm wavelengths. This allows typical analytical sensitivities in the 100-1000 ng range for a number of PAH when using this method. The main advantages of this method are that the required instrumentation is relatively inexpensive and the ready availability of high quality spectrophotometers assures reliable operation, high degree of precision and good reproducibility between laboratories and over extended periods of time. The main disadvantages of the spectrophotometric method are: a) its lower sensitivity compared to the other available methods of detection; b) the requirement for extensive prior separation of individual components for reliable identification and accurate quantitation.

Ideally, quantitative determination is based on single-component extracts. However quantitation of individual components

in a multicomponent mixture may be achieved with a reasonable degree of accuracy if the identity of all components in the mixture has been established by reference to several characteristic peaks or by other means. To be reliable, such determinations must always be based on absorbance measurements at more than one characteristic wavelength. Detection and identification of PAHin some crude or partially resolved environmental mixtures may be possible, based on U.V.-visible spectrophotometric data, with the help of computer analysis of a large number of key bands.

When used in combination with the high pressure liquid chromatographic method of separation,[352,405] quantitation may be achieved either by scanning the entire spectral range of the effluent fractions or by the so-called HPLC peak-height method, in which the U.V. detector device is set at a suitable fixed wavelength. While quantitation in the former case may be based on comparison with published standard values of molar absorptivities, quantitation in the latter case must be based on the actual use of standards under identical HPLC operating conditions. The combination of HPLC-UV method of analysis has resulted in reducing the lower limit of detection for B(a)P to about 10 ng.[352]

5.4.2. Fluorimetry and Other Luminescence Techniques:

The characteristic fluorescence of PAH has been well recognized and used as a powerful tool for their detection by the earliest investigators. Following the development, in the 1950's of improved instruments which overcame such early instrumental problems as random fluctuations in line and source intensity, this method became firmly established as an equally powerful tool

for the quantitation of PAH. However, a number of precautions must be observed and several limitations must be kept in mind.

The main advantages of luminescence techniques relative to absorption techniques are their increased sensitivity and selectivity.

The increased sensitivity is due in part to the more intense light sources used in luminescence measurements, to the generally lower detector noise and partly to the fact that luminescence signals can be amplified directly, whereas in absorption the detector must distinguish a small difference between two large signals. As a result, the sensitivity of luminescence methods is often two to three orders of magnitude greater than that of absorbance methods.[12] Of course, the material to be detected must itself have an appreciable luminescence quantum efficiency, which is indeed the case with most PAH. Thus, linear fluorescence detector responses were obtained with a number of PAH, including BA, Chr, Per, B(a)P, B(e)P, B(k)F, B(g,h,i)Per, in the range of 10 pg to 100 ng.[341,396] The most recently reported detection limits (defined as a signal to noise ratio of 2) ranged from 0.4 pg for B(k)F to 5.1 pg for B(e)P, with 1.1 pg for B(a)P, when HPLC separation was combined with fluorescence detection under optimum conditions.[396] Relative standard deviations of HPLC peak heights were reported to vary between 0.32 and 2.66%.[396]

Perhaps of even greater significance than sensitivity is the improved selectivity of luminescence methods. It is a well known fact that many more types of molecules absorb than re-emit radiation. Consequently there will be fewer inter-

fering species when using luminescence methods. Furthermore, in luminescence, the analyst can select both the excitation and the detection wavelengths so as to maximize the selectivity for one particular compound, whereas no such choice exists when using absorption techniques. The selectivity of the method may be further increased by using two or more excitation/emission wavelength combinations for the characterization of complex environmental mixtures. Examples of the exploitation of this advantage include the quantitative determination of Chrysene and Benz(a)Anthracene or B(a)P and B(k)F in the presence of each other, in cases where no complete separation could be achieved by chromatographic methods. The choice of other factors such as temperature, solvent, added sensitizers or quenchers can also be used to advantage to improve the selectivity of luminescence methods. For instance, B(a)P may be detected and quantitatively measured in the presence of as many as 40 PAH compounds under appropriate conditions.

Various modifications of conventional luminescene techniques have been developed to further improve sensitivity and/or selectivity. These include the use of: 1) low temperature ($77^{o}K$) fluorescence techniques first developed by SHPOLSKII and other Russian workers and later modified by SAWICKI and by KIRKBRIGHT and co-workers, resulting in the appearance of highly structured, quasi-linear spectra; 2) first or second derivative techniques, to emphasize minor features in the spectra; 3) selective modulation of the excitation monochromator at a maximum absorption peak of an interfering fluorescent species, allowing the nulling of the interfering spectrum while the desired spectrum is observed; 4) synchronous luminescence, where the excitation

and emission wavelengths are both varied simultaneously, with a fixed wavelength difference, resulting in band narrowing and simplification of spectra; 5) sensitized fluorescence,[410] used mainly for preliminary screening and semi-quantitative determination, where the inherent fluorescence of traces of PAH is greatly enhanced in the presence of certain sensitizers such as naphthalene, allowing a detection limit of 1-10 pg, detectable visually on filter paper; (without sensitization, the typical in-situ detection limit for most PAH on thin-layer chromatograms has been reported[378] to range from 1 to 100 ng); 6) room temperature phosphorescence,[411] consisting of adsorption of the sample on various rigid, matrix-like supports such as silica alumina, cellulose or filter paper and excitation in the presence of a heavy atom perturber such as silver nitrate, facilitating population of the triplet state through spin-orbit coupling and intersystem crossing. This method obviates many of the difficulties of operating at low temperatures in rigid glass matrices and also allows achievement of picogram detection limits for some PAH such as chrysene, fluoranthene, phenathrene, pyrene, 2-methylnaphthalene; 7) X-ray[412] and laser[413] excitation of PAH have also been proposed to improve both selectivity (due to the high monochromaticity of the existing source) and sensitivity (by increasing the intensity of fluorescence and phosphorescence emissions through larger population of higher energy electronic levels).

While sensitivity and selectivity are obvious advantages of the fluorimetric method of analysis for PAH, it also has several limitations which include the following:

1) instrument characteristics such as detector noise, light scattering, accuracy and repeatability of wavelength settings, fluctuations of source intensity, positioning of sample cell in the optical system all influence the accuracy and precision of emission measurements;

2) since emission intensities are expressed in arbitrary units and their values are largely dependent on instrumental and environmental variables such as temperature, pH, solvents, etc., comparison of spectral data obtained at different laboratories is difficult and requires extensive corrections, strict adherence to, and reporting of standardized conditions, and the constant use of standards;

3) quenching of fluorescence by polar solvents, by impurities, by oxygen and simply by too high concentration of the fluorescent species itself may be sources of serious error and special care has to be taken to avoid them;

4) excimer formation, sensitized fluorescence and photo-oxidation may cause problems in solid state fluorescence measurements, for instance when in-situ evaluation of thin-layer chromatograms is attempted.

5.4.3. Detectors for Gas Chromatography:

The relatively small sample capacity of most GC columns, coupled with the presence of most PAH in trace quantities only, requires the use of the most sensitive detectors for GC analysis of PAH. Flame ionization detectors (FID) are the most widely used ones for this purpose. This device detects the current produced

354, 387

by intermediate ionized products formed by combustion of each eluting species in a hydrogen-oxygen flame. The advantage of FID is its linear response over a wide dynamic range. The FID signal is usually directly proportional to the number of carbon atoms burned and thus the detector response is quantitatively comparable for most PAH. The lower detection limit for PAH is in the low nanogram range with FID.

Another type of detector which may be used for the GC analysis of PAH is the electron capture detector (ECD)[384, 414] which detects eluting species capable of capturing electrons by sensing a decrease in intensity of an electron stream emitted from a beta emitter and collected on a cathode. The advantages of the ECD are its greater sensitivity and selectivity. However these can easily be considered disadvantages as well, under certain circumstances. Thus, the ability of PAH to capture electrons varies with their molecular structure. This allows, for instance, selective detection of B(a)P in the presence of B(e)P,[414] the latter being much less capable to capture electrons than the former. On the other hand, because of the differences in their electron capturing abilities, the EC detector signal must be calibrated for each different PAH for quantitative analysis. While sensitivity of ECD can be an advantage in detecting trace amounts of some PAH, it can also be a major cause of error in the presence of minor components or impurities with very high ECD responses co-eluting with major PAH components. A third type of GC detector, the use of which for PAH analysis has been reported very recently is the photoionization detector (PID).[415] Its sensitivity apparently exceeds that of the FID by a factor

of 10 to 40, with approximate lower limit of detection of 0.05-0.1 ng, and shows little response to water or acetonitrile. This allows the use of isothermal GC conditions resulting in better reproducibility of retention times and a more stable baseline. However, PID has a maximum operating temperature limit of 250°C and gradually becomes fogged, which results in a decrease in sensitivity. Apart from the above-mentioned detectors, specifically designed for GC work, the mass spectrometer is one of the most sensitive GC detectors when coupled with the latter.

5.4.4. Mass Spectrometry -

Mass spectrometry has become another widely accepted method for the detection, identification and quantitation of PAH.[347,355,389] This method is based on high-vacuum ionization of the parent molecules achieved generally by bombardment with high energy (70 ev) electrons so as to dislodge an orbital electron and create positively charged molecular ions. The excess kinetic energy imparted to the molecular ion in this process may be sufficient to rupture the parent ion into smaller ionized fragments. The molecular ions and fragments are then deflected and focussed by electric or magnetic fields and displayed according to their masses, resulting in mass spectra which are characteristic of the original molecules. Alternative ionization methods include low-ionizing-voltage electron bombardment and chemical ionization procedures, resulting in less fragmentation and mass spectra containing mainly singly-charged intact molecular ions. The separation of ionized species according to their masses is accomplished either by high-resolution magnetic scanning instruments[416,417] or lower resolution quadrupole instruments.[389] The latter

type of equipment has a number of advantages, including its lower cost, ease of operation and maintenance, extremely high speed linear mass scans, while at the same time providing adequate resolving power. Because sensitivity and resolution are inversely related, quadrupole instruments also provide the highest sensitivity, affording detection limits of the order of 10^{-11} g.[12] Sample introduction to the mass spectrometer may be accomplished through programmed temperature probe distillation,[417] or by direct interfacing with a gas chromatographic system.[389] As a GC detector the mass spectrometer possess the advantage over UV and fluorescence methods of being able to detect all organic species present, not just the absorbing and emitting ones. This is certainly desirable for complete analysis of complex environmental samples, and for ascertaining the purity of individual chromatographic fractions. As an enormous amount of mass spectral data may be generated even for a single GC run, evaluation requires computerized data processing. Thus, mass spectra of individual GC fractions are encoded in computer -readable form, stored on a disk and recalled whenever desired. The computer output may be in the form of a reconstructed gas chromatogram (total ion current vs. spectrum number), specific ion mass chromatogram (specific m/e abundance vs. spectrum no.) or, for final identification purposes, mass spectra corresponding to specific chromatographic fractions may be recalled from the computer memory.

One problem with electron-impact ionization mass spectrometry is that isomeric PAH may often have identical mass spectra, in which case unambiguous identification is impossible, pointing to the importance of using highly efficient GC columns for

separation. On the other hand, mass spectrometry is particularly suited for the detection of alkyl-substituted PAH compounds. Quantitation is achieved by the use of standard calibration curves, plotting peak ratios relative to internal standards versus quantities of PAH compound used. Daily calibration is recommended for quantitation purposes, due to the temporal variability of instrumental parameters. PAH may be determined this way with relative standard deviations varying between 5 and 10%, and approximate detection limits of about 1 ng.[395]

5.4.5 Other Methods -

Mention should be made here of the application of proton nuclear magnetic resonance (NMR) spectroscopy and of infrared (IR) spectroscopy to the analysis of PAH. Because of their low sensitivities relative to the other techniques discussed above, these methods have seen very little use until recently. However the development of rapid spectrum accumulation through use of Fourier transform techniques and dedicated computer instrumentation have sufficiently increased the sensitivities of both these methods, so as to make them applicable to identification of PAH compounds in small environmental samples.

Fourier transform NMR can be especially useful in identifying the position of substitution in methylated PAH derivatives [355,418] or in PAH metabolites[419], whereas Fourier transform IR has been used[420] for identification of individual PAH compounds in synthetic mixtures as well as in liquified coal and shale oil samples.

Identification of 20 µg PAH in 1 mg sample has been achieved by FT-NMR[418], while typical detection limits for PAH by FT-IR are in the 50 to 200 ng range when using beam-condensing optics.[420]

6. ENVIRONMENTAL CONCENTRATIONS OF PAH:

6.1 General:

Concern about the potentially harmful, and particularly carcinogenic, effects of PAH has encouraged monitoring activities to detect and determine their general environmental concentrations. Such activities became possible approximately since the late 1950's, with the development of sampling and analytical instrumentation of adequate sensitivity. In view of the great variety of natural and anthropogenic sources of PAH, as discussed in Section 2, it is not surprising that their presence proved to be ubiquitious, many PAH having since been detected in trace quantities in air, water, and soil, as well as in raw and processed food.

While knowledge of ambient PAH levels is definitely required for the eventual evaluation of health and other environmental effects due to low level exposure to these compounds, analytical data should be interpreted with caution, for the following reasons.

1) Data given by different studies related to different time periods and different geographical areas may be difficult to compare and to correlate because of the great diversity of sampling, sample handling and analytical methods used. Therefore, standardization of sampling and analytical methodology would be desirable.

2) When trying to correlate ambient environmental exposure levels with cancer incidence, the time lag between exposure and development of cancer will always represent an important element of uncertainty, which should be given adequate consideration.

3) Analytical data on a single ingredient such as B(a)P or even on a few selected PAH components may give a poor picture of the hazardous nature of a complex environmental mixture, due to the known synergistic and possible antagonistic effects that various components have on each other. Therefore, as complete an analysis as possible, preferably coupled with appropriate biological testing, would be desirable.

Keeping these caveats in mind, a review of environmental concentrations of PAH still provides much needed and useful information.

6.2 Ambient Air:

To date, over 100 different PAH compounds have been detected and identified in airborne particulate matter.[355,389] However, quantitative air-quality surveys are usually restricted to a few representative ones for reasons of cost-effectiveness and to assure reasonable precision and accuracy. B(a)P has traditionally been the most extensively monitored PAH compound due to its known carcinogenic properties, and has frequently been used as an indicator for other PAH present. This however is a questionable practice since other PAH such as Chr or B(g,h,i)Per were frequently shown to be present in substantially larger amounts and because the relative concentration of different PAH compounds is known to be highly variable depending on sources and conditions of combustion. Thus, for instance, Coronene is probably a better indicator of automotive PAH sources than B(a)P itself.[421]

Ambient air quality data for PAH have been extensively reviewed in several publications ([12,17,18]), and only a few representative data and general trends will be presented here, and particular reference will be made to available Ontario data.

Early data in the late 1950's and early 1960's were usually obtained by column chromatographic cleanup and U.V. spectrophotometric or fluorometric determination and resulted in establishing the following ranges of ambient levels for a few representative PAH, on a world-wide[18] or U.S.[422] basis:

B(a)P:	winter	0.6-104	ng/m^3
	summer	0.03-4	ng/m^3
	ave. U.S. urban air:	6	ng/m^3
B(e)P:	winter	2.9-208	ng/m^3
	summer	0.01-10	ng/m^3
	ave. U.S. urban air:	5	ng/m^3
BA:	winter	94-361	ng/m^3
	summer	1.6-136	ng/m^3
	ave. U.S. urban air:	4	ng/m^3
Chr:	winter	20-361	ng/m^3
	summer	2.5-3.6	ng/m^3
B(g,h,i)Per:			
	ave. U.S. urban air:	8	ng/m^3
Cor:	ave. U.S. urban air:	2	ng/m^3
F:	ave. U.S. urban air	4	ng/m^3
Per:	ave. U.S. urban air	0.7	ng/m^3

Similar ranges of ambient atmospheric levels were obtained in an early survey[423] of 20 sites in Ontario for B(e)P (0.3-11 ng/m^3), Cor (3-48 ng/m^3) and B(k)F (1.3-14 ng/m^3).

TABLE X

Results of Two-Year (1971-1973) Survey for B(a)P and B(k)F in Ontario [424]

LOCATION	B(a)P* (ng/m^3)			B(k)F* (ng/m^3)		
	Max.	Min.	Ave.	Max.	Min.	Ave.
Welland	116.0	0.06	5.98	176.0	0.43	19.57
Windsor	35.4	0.05	2.19	73.4	0.12	4.14
Hamilton	23.4	0.01	2.38	45.7	0.05	3.65
Sault Ste. Marie	22.5	0.02	0.98	34.0	0.09	1.75
Cornwall	6.7	0.01	0.50	19.5	0.06	1.12
Ottawa	1.85	0.01	0.37	1.86	0.01	0.46
North Bay	15.6	0.03	0.56	7.35	0.03	0.44
Toronto(Downtown)	4.27	0.04	0.58	4.38	0.05	0.73
Toronto(near highway)	1.70	0.05	0.6	2.24	0.09	0.53
Sarnia	4.31	0.01	0.26	5.18	0.04	0.35

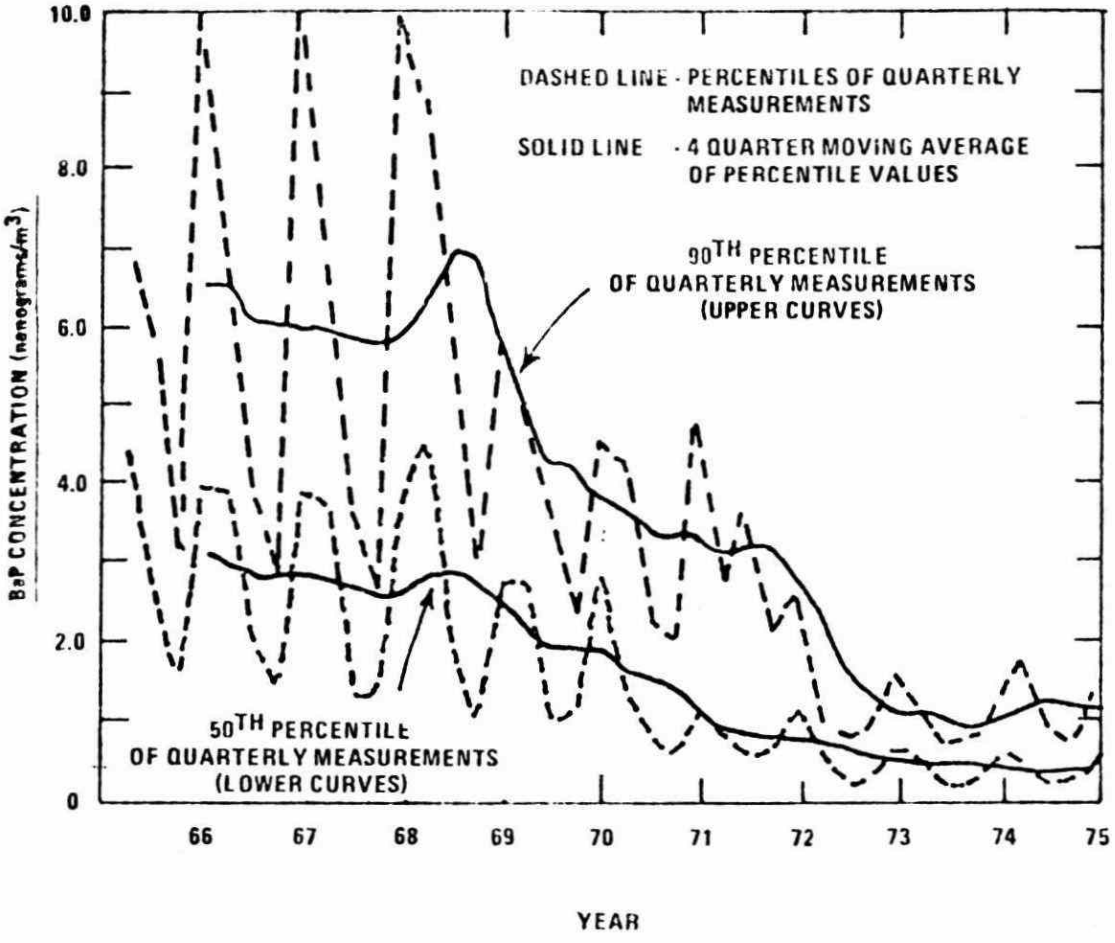
*Analytical method used: column chromatography-fluorimetry.

An extensive two-year survey of 10 Ontario urban sites for B(a)P and B(k)F was conducted by the Ontario Ministry of the Environment in 1971-1973,[424] results of which are presented in summary form in Table X . These show a clear difference in ambient levels found in industrialized and non-industrialized areas. Furthermore the type of industries prevalent in a certain area also has a bearing on the ambient levels of these two PAH, as indicated by the unusually low levels in the petrochemical city of Sarnia as compared for instance to the levels found in the steel city of Hamilton. During the above study, peak levels of these two PAH were found during periods of temperature inversion and significant positive correlation was observed with wind direction, indicating the influence of sources, and, to some extent, with atmospheric dust (total suspended particulate) levels.

Ambient atmospheric concentrations of B(a)P have been extensively monitored by the National Air Surveillance Network (NASN) of the United States and detailed tabulation of data from 250 NASN stations during 1966-1970[17] and from 40 NASN stations during 1971-1975[197] were published. Seasonal and running averages for 34 NASN urban stations, including 24 locations with coke ovens present, are represented on Figure 3, indicating the following significant trends:

1) During the decade of 1966 to 1975 the 90th percentile of annual average B(a)P concentrations at U.S. urban sites dropped from 6.5 ng/m^3 to 1.2 ng/m^3, with the corresponding medians dropping from 3.2 ng/m^3 to 0.5 ng/m^3.
2) Seasonal variations (high winter vs. low summer levels of B(a)P) were much more pronounced during the first half of

FIGURE 4: B(a)P Monitoring Trends at 34 NASN Urban Stations(197)



the decade than during the second half.

These trends are believed to be due to decreased coal consumption for space heating during winter months, to improved controls of coke oven and other emissions and to restrictions on open burning of wastes. Similar trends are expected to prevail in Ontario as well, for similar reasons, as indicated by the generally decreasing trend in levels of suspended particulate matter in the major industrialized areas. Results of a continuing survey of atmospheric PAH levels in Ontario are expected to be available in the near future.

Some more recently published data on atmospheric PAH levels are given in Table XI for comparative purposes.

6.3 Aquatic Environment:

Levels of PAH found in various aqueous systems have also been the subject of several reviews (11, 154). Carcinogenic PAH levels were reported to range from 1 to 100 ng/L in groundwater, from 10 to 25 ng/L in treated river and lake water, from 25 to 100 ng/L in surface water and over 100 ng/L (ppt) in contaminated surface water.[428] Table XII lists concentration data for a few PAH found in various types of water samples at different levels of contamination. Corresponding Ontario data are presently not available. However, SMILLIE and collaborators[429] have recently tested several samples of plant effluents discharged into Hamilton Harbour and found several PAH present at concentrations ranging from 0.02 to 66 µg/L. For instance B(a)P concentration found in several effluent samples ranged from 0.03 to 30 µg/L.[429]

TABLE XI

Recently Published Data on PAH Levels in Ambient Air (Ontario and Worldwide) (ng/m^3)

Location	Year (Season)	Anal. Meth.	B(a)P	B(e)P	Per	B(k)F	B(b)F	B(g,h,i)Per	Reference
Toronto (401)	1975 (Apr.-June)	TLC-Fluor.	.79	.44	.10	.43	.87	5.85	356
Toronto (401)	1975 (Jul.-Sept)	"	1.05	.52	.12	.57	.80	7.13	"
Toronto (401)	1975 (Oct.-Dec.)	"	1.67	1.29	.19	.91	1.39	10.52	"
Toronto (401)	1976 (Jan.-Mar.)	"	.72	.78	.10	.51	.78	4.41	"
Toronto (Suburb)	1975 (Apr.-June)	"	.66	.48	.10	.32	.89	5.07	"
Toronto (Suburb)	1975 (Jul.-Sept.)	"	.41	.37	.06	.28	.69	3.30	"
Toronto (Suburb)	1975 (Oct.-Dec.)	"	.73	.40	.13	.60	1.26	4.69	"
Toronto (Suburb)	1976 (Jan.-Mar.)	"	.81	.79	.05	.52	1.83	9.81	"
Hamilton	1975 (Apr.-June)	"	1.40	.60	.14	.42	.81	5.81	"
Hamilton	1975 (Jul.-Sept.)	"	2.35	1.40	.28	1.42	2.63	7.18	"
Hamilton	1975 (Oct.-Dec.)	"	3.50	3.77	.43	5.14	7.84	7.53	"
Hamilton	1976 (Jan.-Mar.)	"	1.93	1.61	.35	.44	2.30	6.42	"
Sarnia	1975 (Apr.-Jun.)	"	.34	.12	.03	.09	.37	1.04	"
	1975 (Jul.-Sept.)	"	.11	.05	.01	.07	.24	1.05	"

Table XI (Cont'd) - Recently Published Data on PAH Levels in Ambient Air (Ontario and Worldwide) (ng/m^3)

Location	Year(Season)	Anal. Meth.	B(a)P	B(e)P	Per	B(k)F	B(b)F	B(g,h,i)Per	Reference
Sarnia	1975(Oct.-Dec.)	TLC-Fluor.	.60	.60	.09	.44	.94	2.70	356
Sarnia	1976(Jan.-Mar.)	"	.19	.07	.02	.10	.29	1.16	"
Sudbury	1975(Apr.-Jun.)	"	.17	.02	.02	.06	.25	.78	"
Sudbury	1975(Jul.-Sept.)	"	.11	.04	.02	.07	.17	1.10	"
Sudbury	1975(Octo-Dec._	"	.34	.25	.04	.20	.42	2.32	"
Sudbury	1976(Jan.-Mar.)	"	.44	.32	.05	.27	.65	3.01	"
Hamilton 1976 (September-October		HPLC-Fluor.	.92			.04		3.12	143
Sault Ste. Marie 1977 (August)		HPLC-Fluor.	1.19			1.06		5.62	"

Table XI (Cont'd)

Location	Year(Season)	Anal. Meth.	B(a)P	B(e)P	Per	B(k)F	B(b)F	B(g,h,i)Per	P	F	BA	Chr	Cor	Ref.
Antwerp	1976 (Winter)	GC-MS		20.0			26.0		1.1	1.3		11.5		425
Antwerp	1976 (Spring)	GC-MS		3.45			3.7		0.75	0.96		2.05		425
Chacaltaya, Bolivia	1975 (Winter)	GC-MS		0.048			0.046		0.03	0.036		0.052		425
New York City	1975 (Winter)	HPLC-UV	1.15	1.4	0.1	0.6	0.8	0.9	1.05	1.1		3.0	0.4	352
Baltimore Harbor Tunnel, Md.	1975 (May)	HPLC-Fluor.	66.0	69.0				85.0	120.0	93.0	102.0	106.0		341
College Park, Md.	1975 (Apr.)	HPLC-Fluor.	3.2	4.6				3.9	5.2	4.1	4.6	4.8		341
Norway Air Orig. from N. Scotland	1975 (Nov.)	GC-MS	2.05	2.63	0.19		4.01	1.97	3.29	3.96	0.74	3.27	0.18	153
Los Angeles	1974(Mean)	Col.Chr-U.V.	.46	.90	.10	.20	.54	3.27	.45	.31	.18	.60	2.13	421
Budapest	1975	TLC-U.V.	26.8	15.2	11.0			8.6	20.6	10.4	82.1	71.5	10.5	426
London	1976	Col.Chr.-U.V.	7.0	9.0				19.0					11.0	427
Hamilton	1976(Sept.Oct.)	HPLC-Fluor.	.92			.94		3.12	2.09				1.37	143
Sault Ste. Marie	1977(Aug.)	HPLC-Fluor.	1.19			1.06		5.62	1.48	.88				143

TABLE XII

PAH Concentrations Found in Water (ng/L)[18]

Sample Type	BA	B(a)P	B(b)F+B(j)F	IP
Drinking Water	1.0-23.0	0.1-23.4	0.8-11.5	0.1-12.6
Surface Water	4.3-185.0	0.6-114.0	3.7-156.0	1.4-123.0
Sewage Water	31,400.0	34,500.0		15,000.0
Industrial Effluents	25.0-10,360.0	1.0-1,840.0	39.0-9,910.0	17.0-4,980

TABLE XIII

PAH Concentrations Found in Soils and Sediments (µg/kg)[18]

Sample Type	Ba	B(a)P	B(b)F+B(j)F	IP
Forest and Sand	5.0-20.0	0-127.0	15.0-110.0	
Near Highway	1,500.0	2,000		
Near Industry	390	0-939		
Polluted with coal-tar pitch	2,500,000.0	650,000		
River Sediments		190	1,800	600
Marine Sediments	41-330	75-370		

6.4 Soils and Sediments:

Levels of B(a)P in soils have been extensively reviewed by Shabad and collaborators.[430] Data on this and some other PAH have been collated and are presented in summary form in Table XIII.

While soil concentration data in Ontario are lacking, PAH have been shown to be present in Great Lakes sediments,[431] at the mg/kg level.

In general, concentrations of PAH in soil vary depending on the distance from the pollution source, deposition of airborne particulate matter being the main route of contamination. Highest levels of PAH (up to 2.5 g/kg) were found in areas directly contaminated with coal-tar pitch, but the soil polluting influence of vehicle traffic[432] and industrial activities[433] have also been amply demonstrated. Landfills of incinerator fly ash may be an additional significant source of ground-level PAH contamination.[145] However, the fact that PAH have been detected in soils of remote, non-industrialized areas[434] may indicate the extent of contribution of natural sources to global PAH contamination.

6.5 Food:

PAH have been detected and quantitatively determined in a variety of foodstuffs, the ranges of concentration levels found being summarized in Table XIV. The routes of food contamination by PAH may be manyfold: absorption through plant roots from soils is probably the main one in the case of cereals and tuberous vegetables, while deposition of airborne particulate matter and absorption by the waxy coating of leaves is probably more signi-

TABLE XIV

PAH Concentrations Found in Food (µg/kg)[18]

Type of Food	BA	B(a)P	B(b)F	B(e)P	Chr
Smoked Ham	0.4-12.0	0.02-14.6	3.6-15.1	5.2-17.9	0.5-21.2
Charcoal-broiled Meat	1.4-31.0	2.6-11.2		1.7-7.5	0.6-25.4
Smoked Fish	0.02-2.8	0-2.1	37.0	0-29.0	0.3-173.0
Salad	4.6-15.4	2.8-5.3		3.7-14.7	5.7-26.5
Spinach	16.1	7.4		6.9	28.0
Kale	43.6-230.0	12.6-48.1		1.1-19.4	58.0-395.0
Tomatoes	0.3	0.2		0.2	0.5
Vegetable Oils	0.5-13.5	0.9-15.0	0.2-4	0.6-16.5	0.4-16.4
Roasted and Instant Coffee	0.5-14.2	0.1-4.0	1.2-2.1	0.3-7.2	0.6-19.1
Roasted Peanuts	0.95			0.4	0.01-0.71
Cereals	0.4-6.8	0.2-4.1		0.3-4.9	0.8-14.5
Tea		3.9-21.3		1.9-22.3	4.6-6.3
Baker's Dry Yeast		1.8-40.4		3.1-55.0	42.0-203.0

ficant in the case of lettuce, spinach and cabbage. The method of cooking of meat and fish has a significant influence on the amount of PAH present: cooking temperature, time, and whether or not the melted fat is allowed to drop into the heat source are some of the factors determining the concentration of PAH in these foods. Heavy smoking, charcoal-broiling or barbecuing of meat have been shown to increase the amount of PAH present. So does roasting of various grains and nuts such as barley, peanuts and coffee beans.

Corresponding Ontario data regarding PAH levels in local foodstuffs are not available.

While the health significance of ingestion of food and drinks contaminated with PAH at the levels mentioned in Table XIV is much less clear than that of exposure to ambient PAH levels by inhalation, a great deal of caution is indicated nevertheless.

6.6 Coal-tar and Derivatives:

Quantitative data on PAH concentrations in these products, known to cause occupational cancer in humans on continued contact, are presented in Table XV for comparative purposes. Note that these levels are several orders of magnitude higher than any of the ambient concentrations mentioned above.

TABLE XV

PAH Content of Coal-Tar and Derivatives (mg/kg)[18]

PRODUCT	BA	B(j)F	B(a)P	B(e)P	Chr
Coal-tar	up to 6,980	450-630	up to 30,000	1,850-1,880	up to 2,860
Coal-tar pitch	up to 12,500		up to 12,500	5,400-7,000	up to 10,000
Petroleum-asphalt	up to 35		0.1-27	0.03-52	0.4-34
Creosote Oil	up to 2,940	290	$(0.14\text{-}0.2)\times10^{-3}$	150-180	up to 1,340

7. REGULATORY STANDARDS AND GUIDELINES:

7.1 General:

Development of regulatory standards and guidelines for individual PAH is fraught with considerable difficulties, despite the availability of adequate analytical methods and sufficient evidence indicating their potentially hazardous nature, at least in occupational settings. Part of these difficulties stems from the fact that individual PAH usually do not occur separately, but are part of tremendously complex environmental mixtures, the toxic properties of which are difficult to correlate with those of the individual components. For instance the skin cancer incidence in animals caused by exposure to cigarette smoke condensate is 100 times as high as that caused by exposure to equivalent amounts of B(a)P.[204] While extensive animal toxicity (carcinogenicity) data are available on some individual PAH compounds, similar data on complex environmental PAH mixtures are lacking. On the other hand, evidence of human toxicity (carcinogenicity) is based almost entirely on data involving occupational exposures to such complex PAH mixtures as coal tar, soot and coke oven emissions rather than to individual PAH compounds. This makes the extrapolation of animal data to humans, of high level occupational exposure to low level ambient exposures, and the choice of representative PAH compounds to be monitored, that much more difficult. Furthermore, the presently available analytical methods, while adequate in sensitivity, accuracy and reproducibility, are nevertheless not simple and economical enough for routine, widespread use for field monitoring purposes, thus

creating potential problems in enforcing individual PAH standards which might be developed.

It is felt that development of a testing protocol combining rapid and sensitive biological and chemical methods is desirable and might lead to more meaningful, compound-specific regulatory standards for PAH. In the meantime, until more definite and specific correlation can be established between quantitative chemical composition and biological activity of complex PAH-containing mixtures as actually found in the environment, regulatory standards and criteria must be directed towards protecting the public and the environment from excessive exposure to PAH as a class, in terms of the complex mixtures, or certain fractions thereof, that are known to contain them. In addition, certain PAH such as B(a)P may be used as a rough index of general PAH contamination levels. Current standards in use in various countries are usually based on this rationale, even if they do not mention PAH per se. A brief summary of the existing standards and/or guidelines bearing on PAH levels in occupational as well as ambient atmospheric and aquatic environments is given in the following paragraphs, with specific reference to Ontario.

7.2 Occupational Atmosphere:

Occupational standards are usually expressed as threshold limit values (TLV) and refer to airborne concentrations of substances to which it is believed that nearly all workers may be repeatedly exposed, day after day, without adverse effects. Various categories of TLV refer to various time-periods of monitoring. Thus, TLV's may be given as time-weighted average

(TWA) concentrations, referring to concentrations averaged over a normal 8-hour workday, as short-term exposure limits (STEL), referring to maximum allowable concentrations to which workers can be exposed for a period up to 15 minutes, or as ceiling TLV's, referring to concentrations that should not be exceeded even instantaneously. If not otherwise specified, TLV's usually refer to TWA concentrations.

While recognizing that no absolutely safe level can be established for carcinogens, the American Conference of Governmental Industrial Hygienists (ACGIH), in 1967 nevertheless adopted a TLV of 0.2 mg/m^3 for coal tar pitch volatiles (CTPV),[435] described as a "benzene soluble fraction" and aimed to minimize exposure to the following components believed to be carcinogenic: anthracene, B(a)P, phenathrene, acridine, chrysene and pyrene. This TLV was later promulgated as a U.S. Federal Standard under the Occupational Safety and Health Act of 1970.

The same TLV (0.2 mg/m^3) for CTPV was also adopted by the Ontario Ministry of Labor as occupational standard for particulate polycyclic aromatic hydrocarbons (PAH) in Ontario.

A number of other countries, namely Australia, Belgium, Italy, the Netherlands, Switzerland and Yugoslavia, are currently also using the same occupational standard.[436] More recently, the U.S. Occupational Safety and Health Administration promulgated a federal standard on coke oven emissions designed to reduce employee exposure to carcinogenic chemicals, establishing a permissible limit of 0.15 mg/m^3 in terms of benzene-soluble fraction of total particulate matter, as measured with a personal sampler and averaged over 8 hours.[437] The same regulation also mentions that a limit of 0.2 µg/m^3 for B(a)P was recommended by

the Standards Advisory Committee for Coke Oven Emissions, but was not adopted, basically for the same reasons as those mentioned in paragraph 7.1 above. Replacing the "benzene-soluble fraction" with "cyclohexane-soluble fraction" as the basis of standard has also been proposed,[438] in order to eliminate the hazard caused by the known toxic properties of benzene when used as a solvent in the test. This recent recommendation also includes reduction of the organic (cyclohexane)-soluble fraction to 0.1 mg/m^3.[438]

The Soviet Union is presently the only country which has established an occupational exposure limit for a specific PAH compound, namely B(a)P, the current standard having been set at 0.15 $\mu g/m^3$, as maximum allowable concentration (MAC).[439]

7.3 Ambient Atmosphere:

The Soviet Union is also the only country so far, where a maximum allowable concentration has been established for an individual PAH in ambient air. The current standard for B(a)P, based on extrapolation of maximum no-carcinogenic effect level observed in experiments with rodents, is set at 1 ng/m^3.[439] However, no information is available regarding the enforcement record of this standard.

No standards or guidelines for ambient air levels of PAH as such have been established in any other country. However, since the bulk of ambient atmospheric PAH is associated with airborne particulate matter, their concentration in ambient air is expected to parallel that of the latter and consequently, minimizing exposures of the general public to airborne particulate

matter is thought to also result in minimizing exposures to PAH. Standards for suspended particulate matter in ambient air have been established in a number of countries and in Ontario as well. Current ambient air quality criteria for suspended particulates in Ontario call for an annual maximum geometric mean of 60 $\mu g/m^3$, or 120 $\mu g/m^3$ averaged over 24 hours.[440] The corresponding figures in the United States are 75 and 260 $\mu g/m^3$ respectively, established as National Primary Ambient Air Quality Standards, while the desirable secondary standards have been stated as 60 and 150 $\mu g/m^3$ respectively for the annual and daily averages (the latter not to be exceeded more than once a year) of airborne particulate matter.[441] Ontario regulations also call for control of particulate emissions at a level of 100 $\mu g/m^3$, measured as half-hour average concentration at the point of impingement.

Considering that PAH constitute about 0.1% of the airborne particulate matter and that B(a)P forms about 1% of the total PAH usually present in ambient atmospheric particulates compliance to the above-mentioned standards is thought to provide a degree of protection against excessive exposure of the general public to potentially carcinogenic PAH. However, better control of particulate emissions of the respirable size range known to preferentially concetrate PAH, is certainly desirable in order to assure adequate protection of the population at large.

7.4 Drinking Water:

Standards for PAH in drinking water or in other aqueous media have not been ir existence until very recently. However, BORNEFF has stated that about 50 ng/L is the "normal level" for the sum of

six easily measurable PAH, namely fluoranthene (F), B(b)F, B(k)F, B(a)P, B(g,h,i) Per and Indeno (1,2,3-cd) pyrene (IP). He also stated that a total of 100 ng/L for the same six PAH was "acceptable", while a total of 150 ng/L meant that the drinking water was "possibly polluted" and at 200 ng/L it should be rejected.

The World Health Organization has recommended a maximum of 200 ng/L of PAH expressed as the sum of these six easily identified compounds. This recommendation has been adopted by the European Economic Community and similar standards (250 ng/L) are in effect in the Federal Republic of Germany and India. Furthermore, a limit of 30 ng/L was recommended for total carcinogenic PAH, with a limit of 7.5 ng/L set for the B(a)P component of this total. In the drinking water regulations recently introduced by the U.S. EPA, again no specific limits were established for PAH. The Agency opted instead to require the use of best available technology namely treatment with granular activated carbon, to minimize public exposure to potentially harmful chemicals including PAH.

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